Coordinate Variation in Lengths of Deoxyribonucleic Acid Molecules and Head Lengths in Morphological Variants of Bacteriophage T4

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We have investigated three classes of small bacteriophage T4 particles which differ from normal T4 particles in length of their deoxyribonucleic acid (DNA), in head length, in protein content, and in density. The different particles contain DNA molecules measuring 0.90, 0.77, or 0.67, respectively, of the normal T4 length. An additional class of viable particles contains DNA molecules of 1.1 unit length. These discrete differences in DNA length correspond to discrete differences in length (but not width) of the respective heads and are roughly proportional to the resulting differences in head volumes. The measured relative dimensions of the different heads fit best the relative dimensions predicted by a quasi-icosahedral model in which the smallest T4 head corresponds to an icosahedron with a triangulation number $T = 21$. The mid-portion of this structure is thought to be elongated by adding successive rows of gene 23 protein hexamers, the normal T4 head having three added rows. Different mutants produce small particles of the three classes in varying proportions, but no mutant produces exclusively particles of a single class. Particles of each class, with indistinguishable DNA content, show additional minor differences in protein content, as measured by differences in buoyant density and in the relative ratio of ^{32}P to ^{35}S .

Intracellular (vegetative) bacteriophage deoxyribonucleic acid (DNA) molecules are heterogeneous and often greater in length (multimeric) than DNA molecules in mature particles (8, 19, 20, 52). Different phages have developed different ways to cut their vegetative DNA into mature DNA of discrete sizes. It is thought that the cutting functions of phages with nonpermuted base sequences, e.g., λ , recognize certain base sequences (48), while in phages with permuted base sequences, e.g., T4, vegetative DNA is cut to fit the volume of the phage head (52, 54, 55). The following experiments test some of the predictions of the latter model for phage T4.

Populations of bacteriophage T4 contain small proportions of nonviable particles of lower than average buoyant densities, each particle containing a single DNA fragment of fixed length. Although these particles are not viable in single infection, they transmit their genetic information to viable progeny when they co-infect a bacterium with another particle that provides the missing genes. Particles containing DNA molecules which

measure 0.66 of unit length [taking the length of the DNA molecule of a normal T4 particle as unity (43, 44)] have been considered to be identical to the short-headed variants occasionally seen in electron micrographs of T4 lysates (5, 31, 42). The reduction in volume of their heads (as compared with normal heads) corresponds to the reduction of their DNA length, in agreement with Streisinger's "head-full" hypothesis (54). Subsequent experiments revealed two more classes of nonviable particles which contain DNA molecules measuring 0.9 and 0.77 of the unit length (45, 46). The objective of the experiments reported here was to determine whether these different, small, nonviable particles from lysates of the wild type and of different mutants [in particular of the gene 66 mutant E920/96/41 = E920g (16)] have similar properties, i.e., lengths of DNA and protein content, and whether DNA molecules of different lengths are enclosed in heads of different sizes. To facilitate separation of different particles by CsCl gradient centrifugation, we crossed the same *os* (osmotic shock-resistant) mutant with

other mutants and isolated the appropriate recombinants.

Our results show that different mutants produce particles of the different classes (containing DNA molecules of different lengths) in different proportions, but particles from different mutant lysates belonging to each individual class are indistinguishable. In addition to the mutation in gene 66, other mutations, in particular the *os* mutation, influence the proportions of different particles in different lysates.

The discrete differences in DNA length between particles of different classes correspond to discrete differences in head lengths and are found to be proportional to the resulting differences in head volumes. If one considers the three classes of shorter heads as intermediates in assembly, when elongation is prematurely terminated, a model for the arrangement of subunits in the T4 head can be constructed that is also consistent with data of other authors (2, 5, 11, 14, 25, 35, 38, 42).

MATERIALS AND METHODS

Bacteria. All *am*⁺ phage strains were grown in *Escherichia coli* B and assayed in S/6; all *am* strains were grown and assayed in CR 63.

Phage. All *am*⁺ phage strains used were osmotic shock-resistant *os* mutants of T4D which carry the same *os* mutation. The T4D *os* strain was originally obtained from A. H. Doermann. In this paper we shall refer to it as wild type. The *rI* mutant *r48*, the *rII* mutant *r71*, and the *rIII* mutant *r67* have been used previously (45). The mutant E 920/96/41 = E 920g is defective in gene 66 (16). We thank R. S. Edgar for providing this mutant. The gene 66 mutation was combined with the *os* mutation by standard genetic crosses. The strains X1, X2, X3, and X4 are recombinants of wild-type plaque morphology, selected from a cross between T4B *td8* (obtained from E. Simon via R. Werner) and T4D *os ac41*. The *am* strains were originally obtained from R. S. Edgar and have been used previously (45). General procedures and media for the growth of phage stocks and for crosses have been described by Adams (1).

Isolation of osmotic shock-resistant recombinants. The individual stocks were grown from several progeny plaques of an appropriate cross. A portion was saturated with Na₂SO₄. After 30 min, this particle suspension was diluted 1:1,000 into distilled water and plated. New lysates were grown from the survivors and treated the same way. The proportion of survivors in osmotic shock-resistant and -sensitive strains differs by a factor of approximately 10³.

Preparation of labeled stocks. Lysates which were prepared and purified as described (44) were labeled with ³²P (Squibb orthophosphate, 0.2 to 1 mCi per mg of P in the medium) and in some experiments additionally with ³⁵S. (New England Nuclear Corp., Na₂SO₄, 1 to 5 mCi per mg of S in the medium). A 0.79-g amount of solid CsCl (Harshaw, optical grade) was added per 1 ml of purified particle suspension. Each suspension was incubated for 30 min at 45°C

prior to and after addition of the CsCl. Samples were centrifuged in an SW25.1 rotor (8 ml per tube) or in an SW39 rotor (3 ml per tube) at 17,000 or 20,000 rev/min at 23°C for 24 to 48 hr in a Beckman Spinco L2 centrifuge. Fractions were collected through the bottom of the tube. They were diluted 200- to 400-fold. Samples were assayed for viable particles and for radioactivity. When ratios of ³²P to ³⁵S were determined, at least six independent measurements were made of each fraction.

Measurements of lengths of DNA molecules. DNA was extracted with phenol (19). The relative lengths of the extracted DNA molecules were determined from their relative sedimentation through sucrose gradients according to the equation

$$\frac{D^1}{D^2} = \left(\frac{M^1}{M^2} \right)^{0.35} \quad (1)$$

using ³H-labeled DNA molecules from viable particles as reference in the same tube (7). In lysates of *r* mutants, the lengths of DNA molecules in small particles were also determined by a genetic method which is independent of the physical measurements (but depends on transmission of all markers of an incomplete chromosome to progeny) (45). In a typical experiment of this type, *E. coli* B which is restrictive for *am* mutants is simultaneously infected with complete *r*⁺*am* and a single nonviable *r am*⁺ particle. The fraction of incomplete chromosomes (in nonviable particles) which contains the *am*⁺ but has lost the *r* allele can be directly measured as the fraction of bacteria that produces only *r*⁺ progeny and, therefore, gives an infective center of *r*⁺ phenotype. (The other infective centers appear mottled.) When the length of the DNA molecule in the nonviable particle, *L*, > 0.5, and when the distance between the *am* and the *r* mutation, *D*, ≤ (1 - *L*), then

$$L = 1/(Y + 1) + XY/(Y + 1) \quad (2)$$

(where *X* is the size of the functional unit in which the *am* mutation is located). The second term in equation (2) is negligible and *L* can be estimated from *Y*.

Electron microscopy. Grids (400 mesh, PolySciences, Inc.) were coated with a film of 0.3 to 0.4% solution of parlodion (Mallinckrodt) in pentyl acetate (Fisher, chromatograde) and lightly shadowed with carbon in an Edwards high-vacuum evaporator. Phage particles from CsCl gradient fractions were placed on the grids with 50 λ capillary pipettes. After 1 min, the liquid was removed with filter paper. Each grid was quickly rinsed with a single drop of 1% ammonium acetate. Then a drop of 2% phosphotungstic acid (adjusted to pH 7.3 with 1 N KOH) was applied, the excess liquid was removed with filter paper, and the grid was dried and examined at a magnification of 30,000 or 50,000× with an Hitachi HU-11 B electron microscope. At the magnification used, the microscope calibration was checked by measuring negatively stained ferritin molecules (17). Measurements of the phage heads were made directly from the photographic plates by using a Nikon microcomparator with a projection enlargement of 20×.

RESULTS

When osmotic shock-resistant (*os*) T4D phage particles are mixed with a CsCl solution and kept for several days at 4 C, up to 90% of them lose their infectivity. When the phage suspension is heated for 30 min before and after addition of CsCl or when it is kept at room temperature, or both, particles do not lose infectivity. The distribution of samples from the same lysate in CsCl density gradients spun at 4 C with or without previous heating to 45 C (Fig. 1A and B, respectively) shows that infective particles can exist in two states of different densities. The unheated sample contained a minor fraction of particles of lower buoyant density which disappeared after heating, apparently because the particles were converted into the denser state. We have noticed that a larger proportion of particles from the lighter peak as compared with particles from the denser peak is inactivated by rapid dilution out of the CsCl solution. Since in different experiments the proportion of particles in the light peak varied (mostly with time of centrifugation), we chose in all subsequent experiments to heat all suspensions to 45 C when CsCl was added and to spin all CsCl gradients at 23 C.

All further investigations followed the same basic design: we grew lysates in medium containing ^{32}P (or ^{32}P and ^{35}S) and fractionated the particles repeatedly according to their buoyant density. Figure 2A shows a representative density distribution of a wild-type lysate after a first (primary) CsCl gradient centrifugation; Fig. 3A shows the distribution of an E 920g lysate. From particles in different CsCl gradient fractions, we extracted DNA and measured its relative molecu-

lar length (as compared with ^3H -labeled reference DNA of unit length) in neutral sucrose gradients (7).

In the following, we shall describe the DNA molecules in terms of their calculated relative lengths, because the lower sedimentation rate of DNA from the lighter than average, nonviable particles, as compared with DNA from viable particles, is due to differences in length and not in conformation or in base composition (43). The genetic measurements (45) confirm these physical length measurements (Table 1). DNA molecules from infectious particles cosedimented as a sharp band with the reference DNA; DNA molecules from the particles of the lightest buoyant density also sedimented as a sharp band at a rate corresponding to 0.66 of unit length. Most CsCl gradient fractions of intermediate densities yielded upon extraction DNA molecules of several different lengths. However, these molecules did not vary continuously in length, but sedimented as bands corresponding to 0.90, 0.77, or 0.66 of unit length. (The 0.9 value could not be determined as accurately as the other two values.) Different CsCl gradient fractions yielded upon extraction DNA molecules of these different lengths in different proportions. Figure 4 shows two sedimentation patterns which reveal three or four classes of DNA molecules. DNA in B was extracted from pool C, indicated in Fig. 2A. DNA in A came from several combined fractions of another CsCl gradient, not shown here. In general, any single CsCl gradient fraction yielded only one or two classes of DNA molecules. The reasons for finding two classes in some fractions are discussed further below.

FIG. 1. Effect of heating for 30 min prior to and after addition of CsCl on buoyant density of ^{32}P -labeled wild-type particles in CsCl gradients. $\times$ = Viable particles, $\circ$ = ^{32}P . The fractions containing the major peaks in A and B had the same density within the error of the measurements by refractive index; their different position in the gradient is due to differences in average CsCl concentration.

FIG. 2. Separation in CsCl gradients of ^{32}P -labeled wild-type T4 particles differing in density. A, distribution of particles in a primary gradient after centrifugation in an SW 25 rotor; B, C, and D, distribution of particles in secondary gradients, when the pooled fractions marked in A were recentrifuged in an SW39 rotor. $\times$ = viable particles, $\circ$ = ^{32}P .

To achieve better separation of particles containing DNA molecules of different lengths, we pooled several fractions in each of several different regions of the first (primary) CsCl gradient (marked in Fig. 2 and 3) and recentrifuged these particles under conditions similar to those in the primary gradients. In the resulting (secondary) gradients, viable and nonviable particles were better separated than in the primary gradients (Fig. 2B-D and Fig. 3B-E). (The poor resolution in Fig. 2A is due to use of an SW25 rotor and the mechanical mixing during fractionation.)

From the different CsCl gradient fractions, DNA was extracted and analyzed as described before. Many CsCl gradient fractions now yielded mostly DNA molecules of uniform length which sedimented with a rate corresponding to one of the bands in Fig. 4. Figure 5 shows

some representative sucrose gradients. However, some fractions of secondary CsCl gradients still yielded mixtures of DNA molecules of different lengths, in particular those measuring 0.9 or 0.77 of unit length. Recentrifugation of such CsCl fractions resulted in little or no improvement in separation. This suggested that factors other than relative DNA content modify densities of T4 particles. This suggestion is supported by two other observations. (i) Recentrifugation of several fractions, pooled from a primary CsCl gradient, often showed two peaks, differing in density by approximately 0.007, but DNA molecules extracted from the denser or lighter region measured 0.66 of unit length. Replicate sucrose gradients of DNA from the same CsCl gradient fraction showed as much variation in apparent sedimentation rates as did sucrose

FIG. 3. Separation in CsCl gradients of ^{32}P -labeled E 920g particles differing in density. A, distribution of particles in a primary gradient after centrifugation in an SW39 rotor; B, C, and D, re-centrifugations of pooled fractions of decreasing densities from the primary gradient; E, re-centrifugation of a mixture of pools C and D. $\times$ = viable particles, $\circ$ = ^{32}P .

gradients from different CsCl gradient fractions (cf. Table 1). The variability is greater in DNA samples of low radioactivity and can mostly be attributed to statistical errors. (ii) Particles differing in density but containing the same size DNA molecules also showed a different ratio of DNA to protein as discussed below. These observations indicate that density difference

alone is not a sufficient criterion to distinguish T4 particles containing DNA molecules of different lengths. Since we were interested in the correlation of DNA lengths and head sizes, we classified different particles primarily according to the length of their DNA molecules, and we chose for electron microscopy particles from CsCl gradient fractions which yielded DNA molecules of only

TABLE 1. Relative lengths of DNA and ratios of P to S in small particles of different classes

Class ^a	Phage strain ^b	Relative sedimentation rate (D1/D2) of DNA	Normalized average ratio of P to S	Relative DNA length ^c	Relative genome length	Average $\Delta\rho$	
						Found	Expected ^d
II	Wild type	0.965 $\pm$ 0.0105	0.95	0.9	0.89	0.009	0.014
	X1	0.967 $\pm$ 0.0088	0.95				
	(a) E 920g	0.971 $\pm$ 0.010					
	(b) E 920g	0.958					
III	Wild type	0.909 $\pm$ 0.009	0.86	0.77	0.78	0.013	0.028
	X1	0.915 $\pm$ 0.006	0.88				
	(a) E 920g "dense"	0.898 $\pm$ 0.013					
	(a) E 920g "light"	0.893 $\pm$ 0.013					
	(b) E 920 g	0.893 $\pm$ 0.005					
IV	Wild type	0.866 $\pm$ 0.003	0.80	0.66	0.67	0.016	0.042
	X1	0.865 $\pm$ 0.012	0.80				
	(a) E 920g "dense"	0.865 $\pm$ 0.005					
	(a) E 920g "light"	0.861 $\pm$ 0.008					
	(b) E 920g	0.868 $\pm$ 0.009	0.80				
	E 920g rI	0.870 $\pm$ 0.006					
	rII "dense"	0.870 $\pm$ 0.009					
	rII "light"	0.869 $\pm$ 0.015					

^a Note that the data for class II and class III particles probably contain a small error, since these particles cannot be purified as well as class I or class IV particles.

^b (a) and (b) denote two different lysates.

^c The relative genome length in class IV particles from rII and from rIII lysates, as determined by the genetic method, has been reported to be 0.67 of the genome; the relative length in class III particles from the rI strain has been reported to be 0.765 (45). The rI particles used then were not very pure. Crosses involving better purified class III particles from the rII and rIII strains and am mutants opposite on the genetic map to the respective r markers gave an average of 29% bacteria that received the am⁺ but not the r allele from a small particle, indicating that class III particles contain 0.78 of the genome. Similar crosses involving class II particles from rI, rII, or rIII strains gave an average of 12% bacteria that received the am⁺ but not the r allele from a small particle.

^d Expected from the relationship of genome length and density difference in λ particles (3).

FIG. 4. Zone sedimentation of ³²P-labeled DNA (solid line) from particles in two different pooled fractions of intermediate density from a primary CsCl gradient, as compared with ³H-labeled reference DNA (broken lines).

FIG. 5. Zone sedimentation of ^{32}P -labeled DNA (solid line) from particles which were fractionated by repeated CsCl centrifugation. A, from viable particles of higher than average density; B, from viable particles of average density (class I); C, from class II particles; D, from class III particles; E, from class IV particles; F, from particles at the meniscus of the gradient (DNA cosedimenting with reference DNA probably comes from particles that are collected by the meniscus during fraction collection.) Broken line, ^3H -labeled reference DNA.

one length. In this paper we shall call particles containing DNA molecules of 1.00, 0.90, 0.77 or 0.66 length, class I, II, III, or IV particles, respectively.

In some lysates we have found in addition to these small particles viable particles with longer DNA molecules which measure approximately 1.1 of the unit length (Fig. 5A). However, in most lysates these particles could not be fractionated and characterized as well as particles in the three classes of small particles, since they have a similar

buoyant density as some nonviable dense particles. The latter contain unit-length DNA molecules but do not adsorb to bacteria [in Hershey and Chase type experiments (24) not shown here], probably because they lack some essential proteins.

All T4 lysates contain some particles, which float to the top of the CsCl gradient. These particles contain DNA measuring approximately 0.15 to 0.25 of unit length. These particles are probably unfinished heads from which most of

the DNA is digested during purification of the lysate by endogenous or added deoxyribonuclease (23, 34; J. Hosoda, *personal communication*). In premature lysates more than 50% of the DNA is found in such particles.

In similar analyses of several mutants (Table 2), we have found only particles whose DNA molecules are indistinguishable in relative lengths from DNA in the wild-type strain (cf. Table 1).

From each lysate, we have estimated the fraction of particles belonging to each class from the distribution of ^{32}P in the primary CsCl gradient of the total lysate and from the proportions of different DNA molecules (as seen in sucrose gradients) from particles of each CsCl gradient fraction. The results of these estimates are summarized in Table 2. The proportion of different particles varies from lysate to lysate of the same genotype. In our experience, the proportion of small particles increases with age of the infected bacteria and with decreasing glucose concentration in the medium. It also increases during the latent period. However, certain mutations change the proportions of different particles more drastically. The mutant E 920g (16) yields, in addition to class I particles, large proportions of class II, class III, and mostly class IV particles. Lysates of *r48* (*rI*) contain only a few small particles. We have not yet been able to isolate pure class IV particles from this strain. An E 920g *r48* double mutant produces fewer small particles than E 920g alone; in contrast to the single *r48* mutant, most of the small particles are of class IV. Strains X1 through X4 are four different *td8 os* recombinants from a cross T4B *td8* $\times$ T4D *os ac₄₁* (cf. reference 47). They were selected to be *td8 os*; X1 and X3 contain the *ac* mutation; X2 and X4 are *ac*⁺. Strains X1 and X2 (but not X3 and X4) produce more small particles than wild type but fewer than E 920g. Probably, in X1 and

X2, the same site is mutated, but this site has not yet been mapped.

The density difference between particles of classes I and IV has been reported to be less than expected from their difference in DNA content. This indicates that the particles differ also in protein content (16, 23). To measure these differences, we labeled several lysates with ^{32}P and ^{35}S and fractionated them by repeated CsCl gradient centrifugation as described above. From those gradient fractions which contained predominantly small particles of a single class (determined by sedimentation profiles of their DNA molecules), we measured the average ratios of ^{32}P to ^{35}S and normalized these ratios, taking the average ratio determined in normal (class I) particles as unity. These normalized P-to-S ratios are listed in Table 1. In each particle class these ratios are larger than the relative lengths of the respective DNA molecules. Table 1 also shows the average density differences between these gradient fractions and, for comparison, the predicted density differences, if particles of different classes differed only in DNA content, as do λ particles containing DNA molecules of different sizes (3). These data, together with the P-to-S ratios, show clearly that particles containing smaller DNA molecules also contain less protein.

However, more precise calculations on the different protein content are at present meaningless, because superimposed on the difference in P-to-S ratios in particles with DNA molecules of different lengths is a variation of P-to-S ratios and densities among particles with DNA molecules of the same length. Figure 6 shows the distribution of viable particles, ^{32}P and ^{35}S in two representative secondary gradients from a wild-type lysate. All fractions from gradient 6A yielded upon extraction unit-length DNA molecules; all fractions from the major radioactivity peak of

TABLE 2. *Proportion of different particles in different lysates*

Class	Wild ^a type		<i>r</i> I	<i>r</i> II		<i>r</i> III	E 920g			E 920g <i>r</i> I	X ₁	X ₂	X ₃	X ₄	
I	93	94	98	92	90	93	25 ^b	20 ^c	14 ^d	32	76 ^e	65 ^f	66	85	90
II	2	1	1	2	4	2	25 ^b	20 ^c	3 ^d	4	2 ^e	4 ^f	5	4	4
III	<1	1	1	<1	2	2	20 ^b	20 ^c	3 ^d	8	2 ^e	4 ^f	5	4	<1
IV	5	4	<1	5	4	3	30 ^b	40 ^c	80 ^d	56	20 ^e	27 ^f	24	7	5

^a All strains carried the same *os* mutation.

^b Infected when bacteria were at 1.0×10^8 /ml.

^c Infected when bacteria were at 3.5×10^8 /ml.

^d Infected when bacteria were at 8×10^8 /ml.

^e Grown in medium containing 0.05% glucose.

^f Grown in medium containing 0.02% glucose.

FIG. 6. Separation of ^{32}P ^{35}S -labeled particles containing the same amount of DNA but differing in density and protein content in secondary CsCl gradients. A wild-type lysate was fractionated in a primary gradient. Pooled fractions containing mostly viable (class I) particles (A) or mostly class IV particles (B) were recentrifuged to give the results shown. $\times$ = Viable particles, $\circ$ = ^{32}P , $\bullet$ = ^{35}S .

gradient 6B yielded DNA molecules measuring 0.66 of unit length. Nevertheless, in both of these gradients as well as in all other gradients (not shown here), the denser fractions have a higher P-to-S ratio than the lighter fractions (cf. Fig. 6). This fluctuation was unexpected. It must be due to fluctuation in protein content, since the relative sedimentation rates of DNA molecules from "dense" or "light" class IV particles are not significantly different (Table 1) and in any case would not account for the different P-to-S ratios. It suggests that some relatively dense particles lack up to 20% sulfur-containing amino acids, perhaps some of the 18,000-dalton protein described by Forrest and Cummings (18). This difference in protein content among particles with the same size DNA molecules explains the difficulty in fractionating pure class II or class III particles mentioned above.

It is presently not clear and being further investigated whether a fixed proportion of protein may be potentially missing in any particle. This possibility is suggested by the observation that several CsCl gradients (not shown here) gave two peaks of variable particles, differing in buoyant density by approximately 0.007. Similar differences were found among class IV particles. In addition, the density differences between class I and class IV particles in secondary gradients varied: in three gradients the difference was 0.025 ± 0.003 , in six gradients the difference was 0.016 ± 0.002 , and in three gradients the difference was 0.009 ± 0.002 . Undoubtedly oversimplifying the real situation, we can understand these variations if we assume that each class of

particles has a "dense" and a "light" subclass and that some secondary gradients show the density difference between dense class I and light class IV particles (0.025; reference 23), some between light class I and dense class IV particles (0.009), and some between light class I and light class IV or between dense class I and dense class IV particles (0.016; cf. reference 16). When particles are spun at 8 C (although after heating to 45 C), at 32,000 rev/min, probably only "dense" class I and "light" class IV particles are sufficiently separated (cf. references 16 and 23).

The heads of particles containing DNA molecules of known different lengths were measured on electron micrographs. These measurements were mainly done on particles from the E 920g mutant. For comparison, a few particles of other strains were also measured. Figure 7A shows a normal (class I) and a small (class IV) particle from an r_{III} lysate. Figure 7B and C shows unfractionated particles from an E 920g lysate. Figure 8 shows particles which have been fractionated according to the length of their DNA molecules. At first glance, the particles appear to differ in size and shape of their heads, but not of their tails and tailfibers. The measurements of head lengths and width are summarized in Table 3. Since measurements of phage heads from electron micrographs are subject to some inaccuracy due to preparative procedures, calibration of the microscope, and imprecise or biased measurements, all procedures used were standardized wherever possible and particles to be photographed or measured were selected at random. We believe that the observed length differences are not artefacts of the

FIG. 7. Electron micrographs of different *T4* particles. *A*, from a fraction of intermediate density in a primary gradient of an *rIII* lysate; *B* and *C*, from an unfractionated *E* 920g lysate. Magnification, approx. 200,000 $\times$.

FIG. 8. Electron micrographs of fractionated T4 particles of different classes. A, rIII class I; B and C, E 920g class I; D, E 920g class II; E, E 920g class III, F and G, E 920g class IV. Magnification, approx. 129,000 $\times$.

TABLE 3. *Length and width (in nanometers) of T4 heads containing different amounts of DNA*

Class	Phage strain	Total	Length filled	Empty	Total	Width filled	Empty
I	rIII	106.5 $\pm$ 3.3	104.5 $\pm$ 2.3	108.8 $\pm$ 4.4	76.7 $\pm$ 3.3	75.6 $\pm$ 1.3	76.8 $\pm$ 2.7
I	E 920g	108.0 $\pm$ 6.7	108.0 $\pm$ 4.0		78.0 $\pm$ 3.3	78.1 $\pm$ 2.9	
II	E 920g	100.0 $\pm$ 6.7	101.3 $\pm$ 4.5	99.3 $\pm$ 6.9	76.6 $\pm$ 3.3	75.5 $\pm$ 3.8	76.8 $\pm$ 3.5
III	E 920g	91.6 $\pm$ 5.0	91.1 $\pm$ 5.9	93.1 $\pm$ 5.0	76.6 $\pm$ 5.0	74.7 $\pm$ 3.1	79.0 $\pm$ 5.5
IV	E 920g	83.3 $\pm$ 3.3	85.1 $\pm$ 3.9	82.3 $\pm$ 2.2	78.4 $\pm$ 1.7	77.2 $\pm$ 2.7	76.7 $\pm$ 2.7
Unfractionated lysate	E 920g	94.0 $\pm$ 13.0	100.1 $\pm$ 8.8	98.3 $\pm$ 12.4	77.0 $\pm$ 2.0	79.4 $\pm$ 1.6	80.4 $\pm$ 3.5

preparation because random measurements made on unfractionated and fractionated particles belonging to different classes showed a larger statistical variation among the unfractionated particles, whereas the variation in head width remained equal (Table 3). Note also that the average differences in dimensions between heads of class I and class IV particles in Table 3 are nearly identical with the differences between the two rIII heads of different size in one field in Fig. 7A. In the present instance, the measurements were not required to give absolute dimensions, since we were only interested in demonstrating differences between different classes. However, the length of normal (class I) heads (cf. Table 3) is very close to the length of negatively-stained T4 heads (110.7 nm) reported by Luftig (40), who used catalase crystals for calibration, and by Eiserling et al. (16). It is also close to the length of freeze-etched T2 heads (2). Heads which had released their DNA after purification in CsCl gradients show the same dimensions as full heads from the same class of particles (cf. Fig. 8 and Table 3).

DISCUSSION

We have separated three classes of nonviable, small T4 particles from the majority class of viable particles (class I) by virtue of their different buoyant densities in CsCl. We have classified these small particles into classes II, III, and IV according to the lengths of their DNA molecules which measure 0.90, 0.77, or 0.66, respectively, of the unit length DNA in the majority class of viable particles. DNA molecules of class I and of class IV particles are very homogeneous. This is consistent with the observation that T4 DNA molecules of unit length vary only by approximately $\pm 0.6\%$ (33). Homogeneity of DNA lengths in class II and class III particles cannot be proven as rigorously; but homogeneity is likely, since DNA molecules from a mixture of different particles sediment as discrete bands (cf. Fig. 4). There are no detectable major differences between

particles belonging to any one class in different mutant lysates.

The heads of these different particles differ in length, but not in width. Particles containing 0.66-size DNA molecules (class IV) have the isometric heads described previously (5, 6, 16, 42). As the length of the DNA molecules changes in steps of approximately 17%, the length of the heads (but not their width) changes in steps of approximately 10% relative to class IV particles.

Our experiments show clearly that T4 heads with DNA molecules of smaller than unit length are smaller and contain, on the average, less protein than normal heads. Unexpectedly, we found additional differences in protein content among particles of each class (defined by DNA molecules of uniform length). This observation is poorly understood. Potentially missing tail fibers cannot solely be responsible for the observed difference in protein content. It is more likely that one or several of the head proteins (4, 12, 15, 17, 21, 22, 39, 53) are dispensable after the head has been assembled and filled with DNA and that they or the peptides which are cleaved from some of the head proteins (4, 14, 32, 35) remain attached to some but not all heads. Although the reasons remain obscure, it is important to realize that T4 particles containing DNA molecules of indistinguishable length may differ in density due to differences in protein content. We do not know whether these differences are related to reported differences in sedimentation rates (12).

With this minor qualification, our data agree with the hypothesis that the size of mature T4 DNA is determined by the gene(s) which determine the head size (54). They show, in addition, that the gene(s) which determine head size must determine a multiplicity of sizes. This is consistent with the idea that the subunits of the T4 head do not have sufficient information for self-assembly (29, 37) and that different numbers of subunits can assemble to form heads of different discrete sizes. Since the T4 head which can accommodate the whole genome is more compli-

cated than the geometrically simple icosahedron but is nevertheless the most frequently assembled structure in wild-type T4, additional information is necessary to determine its length. Apparently, this information, i.e., the signal to stop addition of subunits to the mid-portion of the head and to start building the caps, is poorly defined. Consequently, a small but finite proportion of heads terminate elongation of the mid-portion prematurely (or too late). Considering the smaller (or larger) than normal T4 heads as such mistakes in assembly can contribute to our understanding of T4 head assembly. The subunits of the T4 head are presently thought to be arranged in a prolate quasi-icosahedral structure (5, 29, 42) or in a twisted bipyramidal hexagonal prism (6, 13). The two classes of heads which are intermediate in size between the isometric and the normal head can be explained within the framework of icosahedral or of hexagonal models, if we assume that the mid-portions of different heads differ in numbers of subunits. In the specific subunit arrangement proposed by Cummings et al. (13), one would have to assume that the overlap of interdigitating subunits can differ in discrete steps. We find it difficult to rationalize this assumption.

Although our results permit no clear distinction between icosahedral or hexagonal prism models, we find it intriguing how well the measured dimensions (head lengths and DNA lengths) of the different heads fit the dimensions of the quasi-icosahedral model discussed in the accompanying note (57) assuming that the head of class IV particles is an icosahedron with a triangulation number $T = 21$ (10). This basic icosahedron is elongated by adding (within the same lattice) successive rows of "capsomeres" (2) (containing hexamers of the gene 23 protein) to the mid-portion but not to the caps (cf. Fig. 1 and 2 of reference 57). Since the elongated T4 heads may be considered as intermediates between spherical and filamentous viruses, one might anticipate that they combine aspects of icosahedral and helical arrangement of subunits. It is pleasing that $T = 21$ predicts a spiral arrangement of "capsomeres."

Growth conditions influence the proportions of small particles in a lysate, but some mutations affect the relative proportions of different small particles more than can be accounted for by variations due to different growth conditions.

So far no mutant has been found that will terminate head elongation definitely at a certain state. This is consistent with the theory that elongation and termination depend on the interaction of several morphopoietic determinants (29). In this respect, our results with the mutant

E 920g require some further comment. Eiserling et al. (16) have investigated this mutant extensively and mapped the mutation in gene 66. They have reported that this mutant produces predominantly isometric heads. Based on this observation, Kellenberger (29) has proposed that gene 66 codes for an elongation factor. We have found considerable proportions of intermediate-sized heads when the E 920g mutation was combined with the *os* mutation. Consistent with this result, E 920g *os* double mutants transmit genome segments of variable lengths to progeny (10, 49). We believe that the different particle distributions in the E 920g *os* as compared with the single E 920g mutant (16) is caused mainly by the additional *os* mutation. *os* Mutations map in gene 24 (Leibo, quoted in reference 37). Laemmli et al. (37) have shown that *am* mutations in gene 24 cause assembly of head proteins of T4 into so-called τ -particles or into polyheads instead of normal heads. (τ -particles are smaller than average heads and do not contain DNA.) Thus it is not surprising that the *os* mutation may affect the accuracy of head elongation. It is also likely that the *os* strain, which we have considered as wild type in the present studies, produces larger proportions of small particles than an *os*⁺ strain. The nature of the mutation in the X1 and X2 strains is not yet known and is being investigated. The influence of the *rI* mutation on the proportions of different small particles is difficult to explain. Possibly it results from the lack of lysis inhibition or indirectly from an alteration in membrane permeability.

Our experiments support the hypothesis that, in phages with circularly permuted DNA, mature DNA is cut from multimeric vegetative DNA to fit the volume of the head (54, 55). However, they do not settle the question whether the head is formed first and then filled with DNA or whether the head condenses around a DNA molecule. Perhaps, DNA condensation and head assembly proceed to some extent simultaneously: as the head membrane is assembled, the capsomeres attach to DNA, rendering the attached portion of the DNA insensitive to deoxyribonuclease. The remaining volume of the head is filled with DNA which is sensitive to deoxyribonuclease until assembly of the completed particle is finished (34). This would explain the presence, in all lysates, of partially filled heads which contain ~ 10 to 25% of the normal DNA length (23, 41, 51; J. Hosoda and E. Mathews, Fed. Proc. **28**: 861, 1969; Fig. 3 and 5). Gene 46 defective mutants, which synthesize only DNA molecules shorter than unit length (26, 50, 51), also produce such partially filled heads (26). Phage P1 probably has circularly permuted DNA molecules like T4

(55). It also forms small morphological variants which contain different amounts of DNA commensurate with the respective head sizes (27, 28, 56). Walker and Anderson (56) have extensively investigated these morphological variants and have explained them as accidents in the assembly of normal structures. In contrast to the T4 variants, all variants of P1 have isometric heads which differ in the size (i.e., number of subunits) of each basic triangle of the icosahedron. As in T4, only one of these morphological variants can accommodate a complete P1 genome, the others contain incomplete genomes. It is remarkable that different phages have developed genetic determinants which exploit different geometric principles to ensure predominant assembly of that head size which can accommodate a complete genome.

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