Encapsidated Conformation of Bacteriophage T7 DNA

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Summary

The structural organization of encapsidated T7 DNA was investigated by cryo-electron microscopy and image processing. A tail-deletion mutant was found to present two preferred views of phage heads: views along the axis through the capsid vertex where the connector protein resides and via which DNA is packaged; and side views perpendicular to this axis. The resulting images reveal striking patterns of concentric rings in axial views, and punctate arrays in side views. As corroborated by computer modeling, these data establish that the T7 chromosome is spooled around this axis in approximately six coaxial shells in a quasicrystalline packing, possibly guided by the core complex on the inner surface of the connector.

Introduction

Chromosomal condensation and decondensation is a fundamental feature of the replicative cycles of organisms ranging from viruses to higher eukaryotes. In general, condensed DNA is transcriptionally quiescent and corresponds to chromosomes in transit. Although it is widely accepted that condensed phase DNA should assume some highly organized configuration that would allow subsequent decondensation to proceed in an orderly manner, the specifics of that configuration have yet to be established for any system. Here, we address that question in the context of DNA packing in bacteriophage heads.

Phage DNA has long attracted interest on account of its seeming tractability, and also because the replicative cycles of the well-studied phages have so much in common (Casjens and Hendrix, 1988) that a solution, once achieved, is likely to have wide applicability. These bacterial viruses have genomes of dsDNA that replicate as linear concatemers, are fed into precursor capsids, and are excised as individual viral chromosomes (Black, 1989). Phage DNA is remarkable for its density of packing—both relative and absolute. For example, in solution, the 40 kbp T7 genome with its contour length of 13.6 μ m might span a space several μ m across and in an infected bacterium, ~1 μ m across. Thus, confinement to

a 55 nm capsid represents a compaction marked by a density increase by a factor of ${\sim}10^4$. The density of encapsidated T7 DNA, ${\sim}450$ mg/ml, is at least 5-fold higher than in metaphase chromatin.

It has long been known, on the basis of a strong reflection at a spacing of \sim 2.5 nm in X-ray diffraction patterns from phage heads (North and Rich, 1961; Gellert and Davies, 1964), that their DNA is packed, at least in part, in aligned bundles. Shape analysis of this reflection indicated the possible degree of ordering, and study of phages with deleted genomes found this spacing to increase as the genome size fell (Earnshaw et al., 1976). However, a structural solution must also specify, among other parameters, the number, size, and connectivity of such bundles and their relationship to the surrounding capsid. Further information emerged from EM studies of DNA released from disrupted phage heads and a variety of other techniques (e.g., Kosturko et al., 1979; Thomas and Serwer, 1990), and diverse models have been proposed. These include the coaxial spool (Richards et al., 1973; Earnshaw and Harrison, 1977; Earnshaw et al., 1978; Harrison, 1983), spiral fold (Black et al., 1985), liquid crystal (Lepault et al., 1987), and folded toroid (Hud, 1995). However, a conclusive structure has yet to be presented for any phage: nor has it been established whether the DNA packing arrangement is deterministic, or whether it varies stochastically from virion to virion.

In recent years, cryo-EM (cryo-electron microscopy) has emerged as a powerful method for visualizing macromolecular structures in their native states (Adrian et al, 1984; Lepault et al, 1987; Booy, 1992). In this study, we have applied this technique to T7, a moderate-sized bacteriophage, whose genome of 39,937 bp is enclosed in a thin-walled icosahedral capsid, ~55 nm in diameter (Steven and Trus, 1986). One of the icosahedral vertices is occupied by the connector protein (Kocsis et al., 1995), which serves as the port via which DNA enters, and ultimately leaves, the capsid. Mounted on the inner surface of the connector is the "core" (Serwer, 1976), a cylindrical protein complex, \sim 26 nm long by \sim 21 nm wide, but otherwise the capsid contains no internal proteins. We have examined T7 DNA in its encapsidated conformation by cryo-EM and computer-aided image processing techniques. This investigation was greatly facilitated by our serendipitous observation that a tailless mutant produces fully packaged heads that preferentially adopt two distinctive orientations in thin ice films, thereby providing two well-defined views.

Results

Tailless T7 Heads Orient Preferentially on EM Grids A T7 mutant deleted for genes 11 and 12 produces DNAfilled heads that lack tails. These heads lose their DNA relatively easily under certain conditions, such as low

relatively easily under certain conditions, such as low salt for extended periods. A mixed field of empty capsids and filled heads is shown in Figure 1a. Empty capsids, delineated by their thin shells, are uniform in size, but



Figure 1. Cryo-electron Micrograph and Computer-Processed Images of T7 Heads from the Complete Tail-Deletion Mutant

(a) Cryo-electron micrograph of a field of bacteriophage T7 heads from the complete tail-deletion mutant. Empty capsids appear as thinwalled particles. Full capsids exhibit the characteristic 2.5 nm spacing of densely packed DNA duplexes in motifs that vary according to viewing direction (see Results). The concentric ring motif (e.g., particle indexed with a closed circle) is the view along the axis through the connector-core vertex and the center of the particle. Particles paired via their connector vertices present side views perpendicular to this axis (e.g., particles indexed with double closed circles). Bar = 50 nm.

(b) Two examples of axial views, at higher magnification. Bar = 25 nm.

(c and d) Images obtained by averaging 21 and 77 particles, respectively. The closed triangle (c) marks the location of the discontinuity between the second and third DNA-associated rings. Also shown in (d) is a scan obtained by azimuthally averaging the accompanying image: it exhibits an outer dense ring (S) corresponding to the protein shell, then at least nine equally spaced DNA-associated rings.

their projected shape varies, depending on the direction from which the capsid is viewed (see below). Full heads are the same size as empty capsids and show the same variation in outline, i.e., in viewing direction. However, density extends across their entire width, displaying distinctive modulations: these take the form of curved striations parallel to the perimeter (the "concentric ring" motif), or punctate arrays and/or linear striations. As noted by Lepault et al. (1987), the contrast with which bundles of DNA duplexes are visualized in cryo-electron micrographs depends on focus. The features described above were consistently observed over a substantial range of defocus values (cf. Figures 2a and 2c).

The most frequent view of tailless T7 heads suspended in thin films of vitreous ice presents a round particle outline and (for DNA-filled heads) the concentric ring motif. We infer that such views are along or close to the symmetry axis that passes from the unique connector vertex through the core and the center of the capsid, and refer to them as axial views, for the following reasons. When projected along a five-fold axis of symmetry, an icosahedron appears round, even one with flat facets (e.g., Figure 1 of Steven et al., 1997). In fact, a flat-faceted icosahedron—as the hexagonal particles show the T7 capsid to be (Figure 1a)—appears round only when viewed from such a direction. This conclusion was confirmed by digitizing a set of such images and determining their orientations by the "common-lines" procedure (Crowther, 1971), which identified them all as being within a few degrees of a five-fold axis. There are six such axes per capsid. That the axis in question is the unique connector-core axis was attested by negatively stained micrographs of heads that showed the core as centered in a majority of cases (data not shown). Since the core is mounted eccentrically on the inner surface of the connector vertex (Serwer, 1976), it appears centered in projection only when viewed along the connector-core axis. Although cores are not distinguished from DNA in low-contrast cryomicrographs of full heads (e.g., Figures 1a, 2a, and 2c), they are visible in micrographs of the T7 prohead, the DNA-free precursor capsid (Figure 2b). For these particles also, the cores are mostly centered, implying axial views.

In addition to round particles (axial views), we also observed many particles with hexagonal outlines, corresponding to views along a three-fold axis. Full hexagonal particles generally exhibited punctate motifs (e.g., Figures 1a and 2a). There are 10 three-fold axes per capsid, of which 5 are perpendicular to the connector-core axis and 5 are inclined at an angle of \sim 35 degrees to it.

The Concentric Ring Motif Observed in Axial Views of T7 Heads

As noted above, axial views of full heads typically present patterns of circular striations, spaced ~2.5 nm apart (Figure 2c). Individual images suggest concentric rings or branched rings or spirals (e.g., Figure 1b) but are too noisy for it to be possible to distinguish unambiguously between these alternatives. To enhance the visibility of the ring system, the images were analyzed by correlation averaging (Figures 1c and 1d). An average of 77 heads revealed a pattern of at least ten concentric rings (Figure 1d). The outermost ring, which is slightly thicker than the rest, represents the protein shell: the other nine rings are equally spaced and presumably represent the superposition pattern of many layers of DNA. A subset of 21 particles also revealed at least nine rings of DNA, but with the appearance of a branch-point between the second and third DNA-associated ring, (at \sim 4 o'clock, Figure 1c).

Interduplex Spacing Increases with Reduced Genome Length

To measure the interduplex spacings in a statistically representative way, we recorded optical diffraction patterns (Figures 2a and 2c) from cryomicrographs of large fields of heads. This experiment was performed for heads containing genomes of three different lengths: full-length (100%), 92.1%, and 84.4%, respectively. The Bragg spacing, determined from the position of the first sharp maximum in the diffraction pattern, was found to increase systematically as the genome shortened (Table 1). We infer that to minimize the electrostatic repulsion between adjacent similarly charged DNA duplexes, they arrange themselves to maximize the spacing between them, subject to the constraint of space available in the capsid interior.

Punctate Patterns Observed in Side Views of Tailless T7 Heads

A second defined view is provided by the observation that tailless T7 particles tend to dimerize via their connectors. In thin films of buffer, such dimers orient themselves with their long axes in the plane of the film, thus presenting a view perpendicular to the connector-core axis (Figures 3a and 3b). In these side views, the packaged DNA projects punctate patterns, sometimes including linear formations. The spacing between adjacent points was \sim 2.5 nm, and somewhat less between adjacent striations, consistent with interduplex spacings measured above (Table 1).

To analyze these patterns for any preferred directionality relative to the connector-core axis, many such images were subjected to diffraction analysis. The images were digitized, and their diffraction patterns calculated after rotating the particles into a defined orientation, i.e., with their connector-core axes vertical (Figures 3c, 3e, and 3f). Many such diffraction patterns were averaged. The resulting composite patterns reveal strong meridional arcs, at an average spacing of ~2.2 nm. The anisotropy of these patterns indicates that their points are not randomly distributed but tend to run in rows oriented within ~20° of the equator (Figures 3e and 3f). Thus, in side views, the duplexes preferentially run transverse to the connector-core axis.

Three-Dimensional Modeling of Spooled DNA

As considered further below (Discussion), the patterns projected in axial views and side views strongly suggest that T7 DNA is wrapped axially in concentric shells, as envisaged by the coaxial spool models (Harrison, 1983). To confirm this interpretation, we used computer modeling to generate a three-dimensional density map corresponding to the envisaged packing geometry, and from it, calculated two-dimensional projections in desired viewing directions. This model is based on several simplifying assumptions: the DNA was wrapped inside a sphere, not a planar-faceted icosahedron, and the DNA duplex was modeled as a unitary thread, \sim 2.0 nm thick. In each shell, the DNA thread was coiled with an axial rise of 2.5 nm per turn, and the diameter of each turn was imposed by the circumscribing sphere. Six coaxially wound shells of DNA were required to accomodate the 13.6 µm T7 genome. Despite these simplifications, the model succeeded in reproducing the main features of the cryomicrographs (Figure 4).

The modeled axial view reproduces the concentric ring pattern particularly well, although we were surprised—in view of the great rarity of this motif for heads other than the tail-deletion mutant-to observe that the rings remain visible when the spool is tilted to guite large angles from the axial direction (Figure 4). For side views, good overall agreement is obtained in the sense that punctate patterns predominate in the model when tilted between about 3° and 15° from this position, and are seen in all the experimental images. In perfect side view, the model gives strong transverse striations that we have not observed in the micrographs: this may mean either that our model assumes an unrealistic degree of geometrical perfection, or that the winding of the spool is other than we have modeled it. It is noteworthy that in cryomicrographs of side views, the DNA is seen to be packed tight against the inner surface of the flat capsid facets (Figure 3). This property, which was not incorporated in our modeling of a spherical spool, implies that the side projection may be expected to vary somewhat, according to viewing position around the





Figure 3. Tailless T7 Heads Paired by Interaction between Their Respective Connectors

Three examples of such dimers visualized by cryo-electron microscopy are shown in (a). (b) shows a dimer that has been penetrated by the negative stain, ammonium vanadate; air dried; and visualized by scanning transmission electron microscopy: the eccentrically mounted internal cores are strongly contrasted. Also, the DNA appears to have retracted from the icosahedral capsid shell as a spherical ball: this feature is not seen in frozen-hydrated specimens and may result from air drying. Cryomicrographs of T7 heads in side view show punctate patterns and/or linear striations that are oriented in a near-equatorial direction (c), or in some cases, slightly tilted from this axis in either direction (e.g., from left to right [e], or from right to left [f]). The connector is at the bottom of the picture in all cases. Also shown are averaged diffraction patterns from 89 particles of the near-equatorial type (d), and from 33 particles of the type shown in (f) with a slight tilt to the right (g). Both show six equally spaced lines radiating from the center of the pattern that represent diffraction from the hexagonal outline of the capsid shell. The DNA spacing is represented in the peripheral ring, which is stronger in the meridional arc, indicative that the striations are preferentially oriented in the near-equatorial direction (d), with only slight but uniform deviations noted, as in (g).

axis. Again, this is a more complex feature than our modeling sought to emulate.

Discussion

Despite the investment of much effort and experimental inventiveness, a conclusive demonstration of the packing geometry of encapsidated DNA has not been made for any bacteriophage. We believe that the observations reported herein constitute such a demonstration in favor of the coaxial spool class of models (Richards et al., 1973; Earnshaw and Harrison, 1977; Earnshaw et al., 1978; Harrison, 1983), as applied to T7.

Orientational Effect of Tailless T7 Heads on EM Grids

The striking "concentric ring" pattern reported here (Figures 1 and 2c) was first observed in cryomicrographs of wild-type T7 by Booy et al. (1992), but only for very few particles. Thus, it was unclear whether these particles represented a rare minority species or a rare view of the majority species. In the tail-deletion mutant used in this

Figure 2. Cryoelectron Micrographs of Purified T7 Heads

(a) and (c) Densely packed monolayers of T7 heads. The particles in (a) are from a double amber mutant in genes 11 and 12 and rarely show the concentric ring motif (an exception is marked with an arrow). The particles in (c) are from a mutant completely deleted for genes 11 and 12, and most of them are preferentially oriented to present the concentric ring motif. These micrographs were recorded at widely different values of defocus: 0.9 μ m defocus (a) and 2.5 μ m (c). Nevertheless, the DNA spacings, represented by the radius of the inner bright ring in each optical diffraction powder pattern (inset in bottom right corners), are the same. (b) shows a field of T7 procapsids that contain no DNA and whose internal cores may be discerned in these projection images. In particles marked with a closed circle, the core appears centered, implying an axial view since the complex is known to be eccentrically mounted (Serwer, 1976), as confirmed in the side view of paired procapsids (double circle). Bar = 50 nm.

Table 1. Hexagonal Packing Spacings for Encapsidated T7				
Genomes				
Genome Length ^a	Bragg Spacing	Center-to-Center Spacin		

Genome Length ^a	Bragg Spacing	Center-to-Center Spacing
100% (full-length)	2.20 nm	2.54 nm
92.1%	2.29 nm	2.64 nm
84.4%	2.38 nm	2.75 nm

^a The amber mutant phage (*11*am37, *12*am3) possesses a full-length genome. The tail deletion mutant (*10A*, Δ *11-12*) with 7.94% deletion was coupled with the 4137 gene *1* deletion (Moffatt and Studier, 1988) of 7.69% to create (4137, *10A*, Δ *11-12*) phage with 84.4% genome length.

study, a majority of heads present this motif (Figure 2c), confirming the latter hypothesis. This observation is of importance in that it indicates that the overall packing arrangement of T7 DNA does not vary randomly from particle to particle but is essentially the same in most if not all heads. This conclusion is supported by our modeling experiments, which demonstrate that the particles with the other observed appearances can be accounted for as different views of the same basic structure.

From this analysis, we infer that the concentric ring motif represents the view along the connector-core axis. This orientational preference most likely reflects the exposure of hydrophobic patches on the surface of the connector that adhere to the air-water interface, thus orienting the particles in thin films of buffer. Dimerization of these heads represents another way of sequestering such patches. Phage with amber mutants in tail genes 11 and 12 do not exhibit the same preferential views (Figure 2a), which may reflect binding of the amber fragments to the connector, annulling its orienting effect.

Axial View: The Concentric Ring Motif

Despite the fact that our model of packed DNA is in some respects an oversimplification, it successfully reproduces the main features of our two defined views: the axial view and the side view. Moreover, these experiments revealed that the ring pattern persists when the spool is tilted to guite high angles, i.e., 20°-30°, from the axial direction. This observation has two implications. First, in a random set of views, the concentric ring motif should be visible on ${\sim}5\%$ (20° cutoff) to ${\sim}12\%$ (30° cutoff) of particles, and thus be much more common than has been our experience. From this consideration, we infer that the views obtained were not randomly distributed but rather, were biased against the axial view. Possibly, this may reflect a tendency of the phage heads to orient with one facet parallel to the substrate or the air-water interface, i.e., viewed close to a threefold axis.

Second, the particles included in our averaged image



The DNA was coiled in six coaxial shells around the connector-core axis. The sequence of images shows how the projection changes in appearance as the viewing direction shifts progressively from 0° relative to the connector-core axis (top left: the axial view) to 90° (side view: bottom right). The DNA is excluded from the space occupied by the core, which in these idealized model images appears as an empty space because this region has not been filled in with "protein density." At about 10° of tilt, the projection appears to develop a discontinuity (whose azimuthal location is marked with a closed triangle) and at about 20°, the projection gives the impression of short straight segments connected at obtuse kink angles.

were selected simply on the basis of their exhibiting the concentric ring motif, and therefore, presumably could cover a considerable range of viewing angles. Interestingly, the branchpoint-like discontinuity between the second and third DNA rings in the averaged experimental image (Figure 1c) is reproduced in the modeled projections when the spool is tilted from the axial direction by \sim 5°–10° (Figure 4). In the latter case, the discontinuity has more the appearance of a buckling: however, we stress that the modeled DNA is smoothly wound and that the apparent discontinuity represents a superposition effect, as it presumably also does in the experimental image. This concordance provides additional support for the coiled spool model as applied to these data. At tilt angles of \sim 20°–30° (Figure 4), circular striations persist in the outer layers, but inside them, the pattern suggests short, straight segments connected by shallow kinks. However, this impression is simply another superposition effect, since the modeled DNA follows a smoothly curved path.

Side Views: The Punctate Motif

The paired heads (Figures 3a and 3b) present side views of packaged T7 DNA. These images typically show punctate patterns, sometimes containing linear striations that usually run in a direction transverse to the connector-core axis. Our modeling shows that the predictions of the coaxial spool are in good overall agreement with the experimental side-view images.

Other Models

Of the many models that have been proposed for packaged phage DNA, we consider that only coaxial spool models (Richards et al., 1973; Earnshaw and Harrison, 1977; Earnshaw et al., 1978; Harrison, 1983) account correctly for our two defined views of T7 heads. The spiral-fold model proposed by Black et al. (1985) for T4, although appealing in other respects, predicts longitudinal striations, not punctate formations plus transverse striations, for the side view. The twisted toroid (Hud, 1995) accounts for neither the axial view nor the side view. However, it is not ruled out that these models may pertain to other viruses. The basic concept of the liquidcrystal model (Lepault et al., 1987) is adaptable to the coaxial spool paradigm with the proviso that essentially the entire genome should form a single liquid crystal in which the duplexes are curved, not straight. We also note that the 2.5 nm spacing of the packaged T7 genome denotes a state of compaction so tight that it should correspond to the 3-D hexagonal crystalline phase of DNA, not the smectic liquid-crystal phase (Livolant and Leforestier, 1996).

There are also chemical cross-linking data to be considered (Widom and Baldwin, 1983; Serwer et al., 1992). These observations indicate that, in a large population of virions, all parts of the genome come into cross-linkable proximity of the protein shell. Harrison (1983) has discussed how these constraints may be met by variations in the winding pattern of coaxial spool models.

In some models, the DNA is envisaged to be folded with sharp kinks rather than wound (Black et al, 1985;

Serwer, 1986; Serwer et al., 1997). However, such kinks have not been observed in DNA released from disrupted phage heads, and Raman spectroscopy of encapsidated P22 DNA was interpreted as not favoring kinks (Aubrey et al., 1992). Accordingly, although our cryoelectron microscopy is not yet sufficiently detailed to rule out kinks—or, indeed, to establish any particular winding pattern for the spool (see Harrison, 1983, for variants)—we would defer further consideration of the kink hypothesis until it is supported by more direct evidence.

A definitive solution would be obtained if one were able to trace the complete three-dimensional path of the 13.6 μ m linear T7 genome in individual virions. Ultimately, this may be possible by electron tomography (Dierksen et al., 1995), although we note that the T7 virion poses a major, possibly unattainable, challenge for this technique, in view of the large number of views (and concomitant radiation damage) needed to reconstruct it to the required resolution (say, 2.0 nm), and degradation of resolution by the limited range of accessible tilt angles.

Spooling of DNA around the Internal Core

The T7 virion has a proteinaceous core mounted on the inner surface of the connector. T7 and its close relative, T3, are the only well-documented phages to possess such a structure. The core has been implicated in capsid assembly (Roeder and Sadowski, 1977), and it may participate somehow in injection of viral DNA into host cells (Garcia and Molineux, 1996), although its precise role has yet to be defined.

Incoming DNA enters the capsid through the connector vertex, i.e., in a direction perpendicular to that in which it is ultimately spooled. It is possible that the core, residing on the inner surface of the connector (see Figure 3b), influences the arrangement of DNA during packaging—for example, by diverting it in a particular direction. In the packaged head, DNA is wrapped around the core, or at least, excluded from the space occupied by it. If a coreless mutant could be found that is easily purified and stable enough to retain its DNA during preparation for microscopy, it would be interesting to ascertain whether it exhibits the same axial spooling of its DNA.

Number of Shells and Packing Density of T7 DNA

In our idealized model, the number of shells required to accommodate the T7 genome (39,937 bp) is six. However, the packing is unlikely to be quite as regular as we have modeled it, so that a seventh partial shell or some DNA in a less-ordered state may occur. In the averaged axial projection, at least nine nested rings of DNA density are resolved (Figure 1e), attesting to the high degree of order in its packing. This observation does not contradict our calculation of the likely number of shells as six because many concentric rings would be seen in projection, even with only two or three shells. This occurs because in a given shell, the DNA is coiled more tightly toward the poles of the sphere and thus appears in projection as multiple rings at lower radii, and also because there is an offset of one duplex diameter between successive shells. As yet, we do not know what

the exact number of shells is. As noted above, there could be more than six. On the other hand, fewer shells surrounding an inner region of less-ordered DNA would generate a similar axial projection. However, the space available in the T7 capsid is fully occupied, with the genome organized in hexagonally packed duplexes with a spacing of 2.5 nm, as in the coaxial spool, and little space is left over for less efficient packing of disordered DNA.

DNA Packaging

Packaging of T7 DNA consists of the following steps. The DNA right end of a concatemer is selected, and packaging into the prohead is initiated (Chung et al., 1990). DNA is translocated in at the phenomenal rate of 140 bp/s at 25° C by the action of terminase at the expense of 1 ATP hydrolyzed per 1.8 bp packaged, (T3: Morita et al., 1993). After ~25% of the genome has entered, the prohead undergoes its maturational expansion and the scaffolding protein is expelled (T3: Shibata et al., 1987). Finally, the terminase recognizes and cuts at the left-end cleavage site, the fully packaged head is stabilized, and the tail assembles on the outside of the connector.

Little is yet known about the configurations assumed by partially packaged genomes. (We stress that the sequence in which our 3-D model of the spool was generated was simply a mathematical convenience: it is not meant to reflect the actual process of DNA packaging, nor the progression of partially packaged states.) In general, we would expect the intrinsic stiffness of the DNA duplex to favor the maximal allowable radius of curvature. Other factors include the mutual electrostatic repulsion of duplexes and physical contraints imposed by the confining capsid walls. In this context, it is instructive to compare the 14% deletion mutant with the wild type, assuming that the conformation of its DNA is the same as that of the wild-type when 86% packaged. Our micrographs indicate that for this intermediate, the concentric spool is already well-established, with a calculated number of five complete shells. As the last 14% (1.9 µm) of DNA enters, approximately half would be laid down in a sixth shell and the remainder ratcheted into a tighter packing of the five pre-existing shells. Less evident but of particular interest are the configurations assumed earlier in packaging, in which the DNA must somehow be readied to enter the spooled state.

Packaged State of DNA in Other Viruses

The only other defined view of a phage head observed by cryo-EM has been the side view of giant T4 heads (Lepault et al., 1987): in their mid-sections, the DNA runs parallel to the long axis. However, it was not determined whether this property is shared by normal prolate T4 heads or whether their DNA bundles are simply oriented parallel to the closest capsid wall. Nevertheless, the simplest assumption is that in wild-type T4, DNA is packed in essentially the same way as in giant heads. If so, it may be that T4 DNA conforms to a spooling arrangement like in T7, but with the spooling axis perpendicular to the orientation observed in T7. At present, however, other models are still viable candidates for T4.

Other phage heads for which a similar spacing has been measured by X-ray diffraction are lambda and P22 (Earnshaw et al., 1976, 1979), and for which comparable motifs have been detected by cryo-EM are lambda and T4 (Lepault et al., 1987). However, it is not clear that the overall ordering of their DNA is the same as in T7. While our hypothesis that it is exposure of a hydrophobic patch on the connector that is responsible for orienting taildeleted T7 heads remains to be proven, it does predict the possibility of similarly orienting other phage heads for cryo-EM analysis by deleting their tails and/or appending hydrophobic moieties to their connectors. If other connectors turn out not to have the same effect, the report that connectors may be exchangable between phages (Donate et al., 1990) suggests the possibility of implanting the T7 connector in chimeric phages for this purpose.

Experimental Procedures

T7 Growth and Capsid Purification

Tailless T7 heads were isolated from lysates of E. coli BL21 cells infected with the T7 complete deletion mutant (10A, Δ 11-12) or the amber mutant phage (11am37, 12am3), as indicated. The lysates were treated with DNase I (10 μ g/ml), supplemented with NaCI to produce a concentration of at least 0.5 M, and clarified by lowspeed centrifugation (10,800 imes g, 10 min). The capsids were precipitated with 10% PEG-8000, centrifuged at 10,800 \times g, 10 min, extracted from the pellet with 1 M NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and banded by isopycnic centrifugation in a buffered CsCl step gradient, as described (Cerritelli and Studier, 1996a). The capsids were collected from the centrifuge tube and rebanded to equilibrium, one time for 11am37, 12am3 or two times for 10A only, Δ 11-12 in a solution consisting of 1.3 volumes of 62.5% CsCl and 1 volume of 10 mM Tris-CI (pH 8.0), 1 mM Na₃EDTA. Prior to examination in the microscope, the capsids were extensively dialyzed against 0.1 M NaCl, 2 mM Tris-HCl (pH 8.0), 0.2 mM EDTA using the filter-float dialysis technique (Cerritelli and Studier, 1996b).

Cryoelectron Microscopy

Vitrified thin films containing T7 heads were prepared from drops of purified suspensions at ~3 mg/ml in a buffer consisting of 0.15 M NaCl, 2 mM Tris–Cl (pH 8), 0.2 mM EDTA, essentially as described by Booy et al. (1985, 1991), except that holey carbon films were used as substrate. Micrographs were recorded relatively close to focus, with the first zero of the phase contrast transfer function (CTF) in the ranges indicated, using a Philips CM20-FEG electron microscope (Philips, Eindhoven, Netherlands), operating at 120 keV and equipped with a Gatan 626 cryoholder (Gatan, Pleasanton, CA). Low-dose techniques were employed, with each exposure corresponding to ~5 electrons/Å². The micrographs were assessed for resolution and stigmation by optical diffraction, and their defocus values were estimated from the positions of the zeros in the contrast transfer function (Lepault and Leonard, 1985).

Image Processing

Micrographs were digitized on a Perkin Elmer 1010MG flat-bed scanner using a scan step and aperture size corresponding to 0.47 nm at the specimen. Particles were extracted and processed using the automated procedure of Conway et al. (1993). Twenty-one particles that displayed the concentric ring motif with particular clarity were analyzed by the "common lines" procedure (Crowther, 1971; Fuller, 1987; Baker et al., 1988, 1989; Trus et al., 1992), and their viewing orientations thus confirmed to be close to a five-fold axis. Correlation averaging was performed using the PIC program (Trus et al., 1996), essentially as described by Cerritelli et al. (1996).

Modeling of Spooled DNA

A FORTRAN program was written to model the coiling of the T7 genome inside the capsid. In the model, a mathematically generated

thread (the DNA) is coiled in successive shells inlaid in a sphere. DNA is specifically excluded from the core, modeled as an internal cylinder, 26 nm in length and 21 nm in diameter. The curve was parameterized and the x, y, and z values at each point were calculated. The packing is made up of shells of DNA, fitted inside of each other. The thread of DNA density starts at the top of the spherical shell and follows a spiral path along the inner surface. Each coil represents an axial advance of 2.5 nm, i.e., there is a pitch of 2.5 nm, but the diameter of each coil depends on its axial position inside the sphere. When the thread reaches the bottom, it continues in the same direction and winds back inside a spherical shell of smaller diameter, i.e., of diameter reduced by the previous shell. The coordinates of each point along the growing thread were registered as voxels of positive density in the three-dimensional map. The corresponding element in the array was scored with a one, a two, or a three to reflect the presence of the DNA density. After each complete turn, a function to calculate the length was called, keeping tally of the total length of the spool. The completed array was then written out as a three-dimensional map file, similar to those used in studies of virus capsids and compatible with the EMMAP programs to generate two-dimensional projections and other visualizations (Baker et al., 1988, 1989; Trus et al., 1996).

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