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Ion etching of bacteriophage T4: Support for a spiral-fold model of packaged DNA

(virus structure/morphogenesis/DNA structure)

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ABSTRACT **Ion etching of bacteriophage T4 erodes virus components progressively from the outside to the inside while preserving the overall structure. The terminal portion of the T4 DNA molecule packaged can be specifically radiolabeled and was found to be eroded more rapidly than the remainder of the DNA. This strongly suggests that the first DNA to enter the prohead is condensed in the center of the capsid and is therefore shielded from the ion beam by the surrounding last packaged DNA. The results support a "spiral-fold" model for the arrangement of DNA within the icosahedral bacteriophage head. According to this model, phage T4 DNA strands run parallel to the long axis of the phage, with sharp (180°) bends at the top and bottom of the capsid. The folds themselves are arranged radially about the long axis of the head in spirally organized shells.**

The arrangement of the nucleic acid within even the simplest of icosahedral viruses is poorly understood (1). In the head of complex, icosahedral bacteriophages, DNA is apparently condensed into a highly ordered structure within a preformed prohead (2). It is believed that the structure of this DNA as well as the mechanism of its packaging are basically the same among all such bacteriophages (3). How DNA is packaged in this injectable form is of interest for higher-order DNA structure; it may also be relevant to the mechanism of chromosome condensation. The currently favored model for the structure of condensed phage DNA, the concentric shell model (Fig. 4*b*), was derived largely from electron microscopic (4) and low-angle x-ray scattering data (5); however, such data are difficult to relate to resolved DNA strands, and there is also evidence opposed to this model (see *Discussion*).

Recently, a new approach, ion etching, has been developed to probe the internal structure of complex viruses (6, 7). Direct application of this technique to the electron microscopy of bacteriophages has, so far, revealed little internal substructure (ref. 6; unpublished observations). However, in addition to direct imaging of the internal structure, this technique allows an examination of the kinetics of breakdown and removal of components from the outside toward the inside of the virus particle. A procedure to radiolabel specifically the terminal portion of the phage T4 DNA to be packaged (8) has made it possible to employ ion etching to ask the following question: If DNA is arranged in a highly ordered structure, is the first DNA to enter the head arranged around the periphery of the head or is it found in the center? The answer to this question allows a reevaluation of the various lines of evidence for the concentric shell model of packaged phage DNA, and it leads to a proposal for a different model.

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MATERIALS AND METHODS

Preparation and Purification of Radioactive Virus. The DNA of phage T4 packaging mutant 49(tsC9)-t(amA3) was labeled uniformly or terminally with D-[^{3}H]glucose or uniformly with [^{14}C]thymidine by growth in the conditionally glucose-deficient host DF2000 as described (8). D-[6-^{3}H]glucose (20 Ci/mmol; 1 Ci = 37 GBq) and [2-^{14}C]thymidine (50 mCi/mmol) were added to the growth medium at concentrations of 30 μCi/ml and 1.5 μCi/ml, respectively (250 μg of deoxyadenosine per ml was also added in [^{14}C]thymidine incorporation experiments). Phage labeled predominantly in the internal proteins were prepared by infecting *Escherichia coli* BE in M9 at 37°C with T4 and adding a mixture of ^{14}C-labeled amino acids (3.5 μCi/ml) from 1 to 4.5 min after infection. Structural proteins were selectively labeled by the same procedure except that radioactive amino acids (0.5 μCi/ml) were added from 15 to 30 min after infection. All radioisotopes were purchased from ICN. Phage were purified by sedimenting the infected bacteria for 12 min at 6000 × *g*, incubating bacterial pellets with pancreatic DNase (25 μg/ml) in the presence of chloroform for 20 min at 37°C, and centrifuging, first at low speed (5 min at 2000 × *g*) and then at high speed on CsCl band gradients (9). Phage employed for some experiments (Fig. 3*a*) were further purified by equilibrium centrifugation on CsCl gradients. Glutaraldehyde fixation was carried out following the low-speed centrifugation by incubating phage in the presence of 2% fresh electron microscope grade glutaraldehyde (Polysciences, Warrington, PA) for 30 min at 4°C, followed by CsCl centrifugation. After CsCl centrifugation, the phage band was removed from the CsCl gradient with a hypodermic syringe and dialyzed first against 10 mM Tris, pH 7.4/1 mM $MgSO_4$/250 mM NaCl and then dialyzed thoroughly against 10 mM Tris, pH 7.4/1 mM $MgSO_4$ (unfixed preparations) or against 100 mM NH_4HCO_3, pH 7.9/1 mM $MgSO_4$ (fixed preparations). Phage preparations were then brought to the same titer (1×10^{10} per ml) or OD_{260} (for fixed samples) with nonradioactive phage prepared at the same time. D-[^{3}H]glucose-labeled phage preparations contained 5000–40,000 cpm of ^{3}H per 5 μl.

Ion Etching. Ion etching was carried out with 5-μl aliquots of radioactive T4 preparations that were spread evenly on glow-discharged 1.3 cm × 1.3 cm squares of aluminum foil (Reynolds 625). After spreading, phage samples were frozen immediately in liquid nitrogen and dried under vacuum overnight. Uniformly and terminally labeled phage preparations were etched side-by-side in Ar^+ plasmas produced in a Technics Hummer V sputter coater operated at 1 Ma, 200 V, and an argon gas pressure of 100 millitorr (1 torr = 133 Pa). The beam was pulsed (5 sec on, 3 sec off) to avoid heating. Following etching for various periods, foil squares were immersed in a toluene-based liquid scintillation fluid (Research Products International, Elk Grove Village, IL) and

assayed for radioactivity in a Packard model 3320 counter. Experimental points reported represent the average and the extremes of results obtained from 5 to 10 identical samples at each ion dose.

Other Methods. Electron microscopy of Ar^+-etched T4 was carried out with phage samples that were lyophilized onto standard electron microscope grids, etched, and shadowed with Pt:Pd (80%:20%) as described (6). $NaDodSO_4$/PAGE of ^{14}C-labeled amino acid-labeled phage proteins was carried out on linear 10–18% polyacrylamide slab gels (9), which were dried and autoradiographed on Kodak XAR-5 film.

RESULTS

Morphological Examination of Etched T4. Ar^+ etching followed by Pt:Pd shadowing and electron microscopy revealed that intact T4 particles were more or less uniformly eroded by the ion beam. Under these Ar^+ plasma-etching conditions, the particles were eroded at essentially the same rate from the bottom and sides as from the top (unpublished observations). The basic structural integrity of the particle was maintained even after long periods of etching, as shown in Fig. 1 *a–c*. During short periods of etching (Fig. 1*b*) the tail fibers were seen to be the first structures lost. After further etching, the tails disappeared almost completely and the heads were much reduced in size (Fig. 1*c*). Even after the longest period of etching examined (10 mA·min), however, the heads retained a generally icosahedral shape and appeared full. Electron microscopic observation, therefore, suggests that even during prolonged etching the phage does not suffer gross morphological change (e.g., breaking of the head–tail junction, emptying of the head) and the DNA retains its overall condensed configuration. Similar observations have been reported (7).

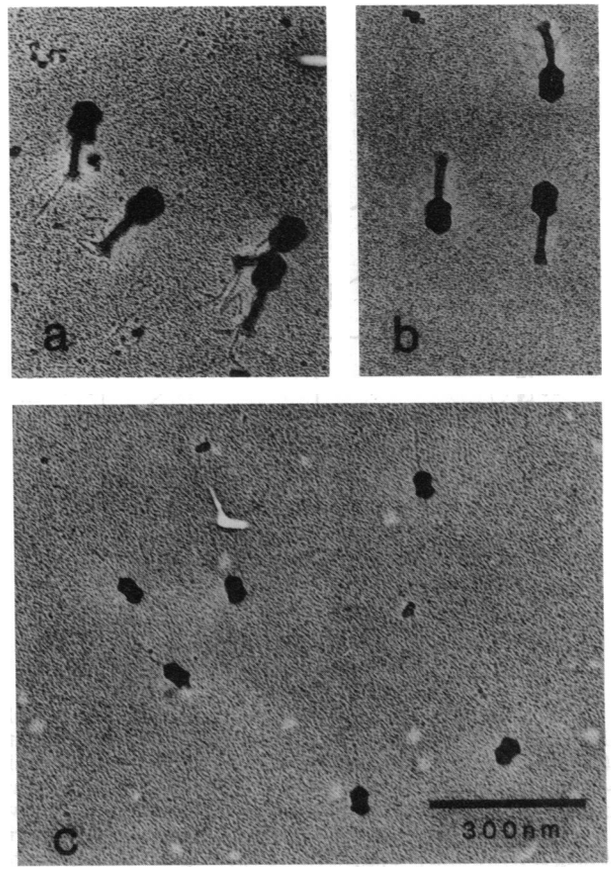

FIG. 1. Electron microscopy of Ar^+-etched bacteriophage T4. Phage particles were frozen in liquid nitrogen and lyophilized prior to ion etching. Following etching, phage samples were shadowed with Pt:Pd and photographed in a JEOL 100cx electron microscope. Micrographs, which are all printed at the same magnification, show control, unetched phage (*a*), phage etched for 1.7 mA·min (*b*), and phage etched for 10 mA·min (*c*).

Rate of Internal and External Viral Protein Erosion. Biochemical evidence also supports the view that Ar^+ etching erodes T4 components sequentially from the outside toward the inside of the particle. A direct test has involved comparison of the etching rates of internal and external head proteins. T4 contains ≈1100 molecules (about 5% of the protein) of three basic, DNA-binding internal proteins (iP1, iP2, and iP3), which are synthesized both early and late after infection. In contrast, the predominant surface structural proteins of the virus are synthesized at late times only (10). It is, therefore, possible to prepare T4 with radioactivity selectively incorporated into either the internal or the external proteins. Fig. 2 *Inset* shows an analysis, by $NaDodSO_4$/PAGE and autoradiography, of T4 proteins prepared from phage labeled early (lane A) or late (lane B) after infection. Radioactivity can be seen to be incorporated selectively into internal proteins during the early labeling period (1–4.5 min) and into external proteins, primarily gp23, the major capsid protein, during the late labeling (15–30 min after infection).

Ar^+ etching of phage labeled selectively in internal or in external proteins revealed that radioactivity was lost more slowly from internal proteins, as shown in Fig. 2. In fact, internal protein label appeared to be lost at a rate comparable to that of DNA (compare Fig. 2 with Fig. 3 *a* and *b*), suggesting that the T4 internal proteins are located within the DNA condensate rather than, for example, in a shell located directly beneath the capsid. The experimental results clearly support the view that etching takes place progressively from the outside toward the inside of the particle and that the basic

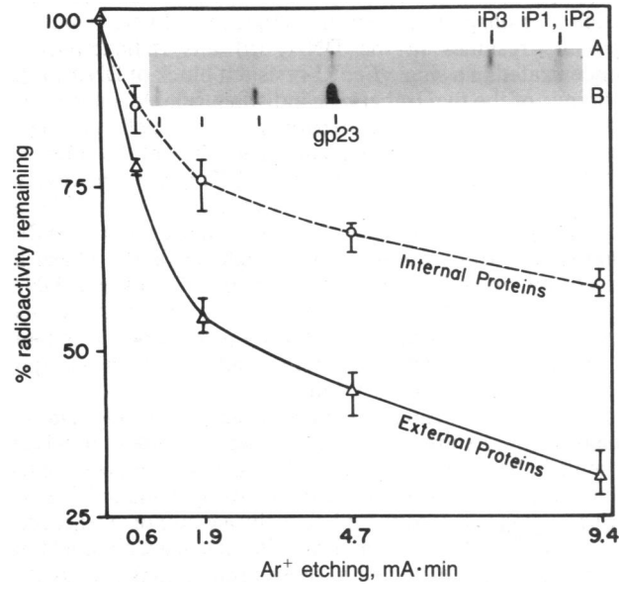

FIG. 2. Comparative etching rates of internal and external proteins in bacteriophage T4. Phage were labeled with ^{14}C-labeled amino acids predominantly in either the internal proteins (iP1, iP2, and iP3) or in the external structural proteins (mainly gp23, the major capsid protein). (*Inset*) $NaDodSO_4$/PAGE analysis of the two phage preparations. Lane A, internal proteins labeled; lane B, external proteins labeled. Selectively labeled phage were fixed with glutaraldehyde and etched with Ar^+. After etching the amount of radioactivity remaining on the support was determined by liquid scintillation spectroscopy and expressed as a percentage of the original amount.

structural integrity of the head is maintained throughout the etching period.

Experimental Design For An Ion-Etching Study Of Packaged DNA Structure. If T4 is eroded progressively from the outside to the inside of the particle, as is suggested by the morphological and biochemical experiments described above, then DNA on the exterior of the phage condensate should be lost before DNA packed at the center of the particle. Therefore, ion etching should allow the geometry of the packaged DNA to be probed if we are able to distinguish particular regions of the DNA. In fact, the last portion (the terminal half, on average) of the T4 molecule to be transferred into the prohead can be specifically radiolabeled (8). The rate at which this specific label is lost during Ar^+ etching can be compared to the rate of loss of the same label distributed uniformly along the entire DNA length. Radioactivity should be lost more slowly from terminally labeled phage if the last DNA packaged is found at the center of the head and more rapidly if it lies near the surface.

The most significant feature of our experimental strategy is the use of phage containing "terminally labeled" DNA. Radioactive label in such phage is present as [^{3}H]glucose molecules attached to hydroxymethylcytosine residues. Selective incorporation of label into the terminal end of the packaged DNA is accomplished by use of a temperature-sensitive mutant phage, 49(tsC9), reversibly temperature-arrested in DNA packaging (11). At the restrictive temperature only the first portion of the DNA is transferred into the prohead, and numerous partially filled heads accumulate *in vivo*. By use of a host conditionally defective in UDP-glucose synthesis, one can, by addition of glucose, fully glucosylate the unpackaged portion of the DNA, whereas the already packaged DNA is not susceptible to glucosylation. Following temperature shift, which removes the block in packaging, virus is produced that is glucosylated only on the terminal portion of the packaged DNA. Genetic and restriction enzyme analysis of such preparations has established that the DNA has the following properties: (*i*) all ^{3}H-label incorporation into phage is present in [^{3}H]glucosyl-hydroxymethylcytosine residues in the DNA; (*ii*) this radioactivity is concentrated in a single *Eco*RI-resistant block of DNA in the terminus of the two (otherwise indistinguishable) ends of the T4 DNA molecule; (*iii*) the length of the terminally packaged segment is distributed about a mean of ≈50% of the entire T4 DNA; and (*iv*) essentially all of the (17%) hydroxymethylcytosine residues in this terminal block are glucosylated. Phage containing terminally labeled DNA do not appear to differ significantly from wild-type (or nonglucosylated) phage in infectivity, particle stability, or amount of packaged DNA (8). Production of phage whose DNA is uniformly labeled with [^{3}H]glucose is accomplished by growth of the mutant phage 49(tsC9) under permissive conditions with incorporation throughout the infection.

Rate of DNA Erosion in Terminally and Uniformly Labeled Phage. Fig. 3*a* shows the results of an experiment in which terminally and uniformly labeled phage were etched under identical conditions. Phage particles were irradiated following spreading at a density far below (by a factor of at least 100) that required to form a monolayer. Particle–particle shielding is, therefore, not expected in these preparations spread at a constant particle concentration. It was consistently observed that ^{3}H label was removed more rapidly from terminally than from uniformly labeled phage. The maximum difference in rate was ≈2-fold and comparable results were obtained with unfixed and with glutaraldehyde-fixed virus preparations (Fig. 3 *a* and *b*).

It was of concern that terminally and uniformly labeled phage preparations be comparable in structural integrity and DNA content. Evidence that this was the case was obtained from the following control experiments. (*i*) Electron microscopic analysis of negatively stained preparations demonstrated that >90% of phage had filled heads. Terminally and uniformly labeled virus did not differ systematically in this respect. (*ii*) Less than 3% of the [^{3}H]glucosyl label was solubilized when phage were treated with pancreatic DNase I. (*iii*) DNA isolated from phage by phenol extraction was eroded at a much higher rate than DNA in intact virus (data not shown).

Finally, as an independent control over particle stability and differential etching rates, the following double-label experiment was performed. Two T4-49ts phage samples were prepared with [^{14}C]thymidine incorporated uniformly into the DNA. One, however, was also labeled uniformly with [^{3}H]glucose, whereas the other contained terminal [^{3}H]glucose label. In the two preparations the [^{14}C]thymidine etching rate was designed to serve as an internal control for the stability of the phage. During etching, the rate of change of the ^{3}H:^{14}C ratio should reflect the relative geometrical accessibility of glucose in DNA to thymidine in the same packaged DNA.

Fig. 3*c* shows the results of experiments in which doubly labeled phage were etched in the Ar^+ beam. They show that the ^{3}H:^{14}C ratio dropped more rapidly when [^{3}H]glucose label was incorporated terminally (T/U) compared to uniformly (U/U) along the DNA. They, therefore, support the results of the single-label experiments in suggesting that the last

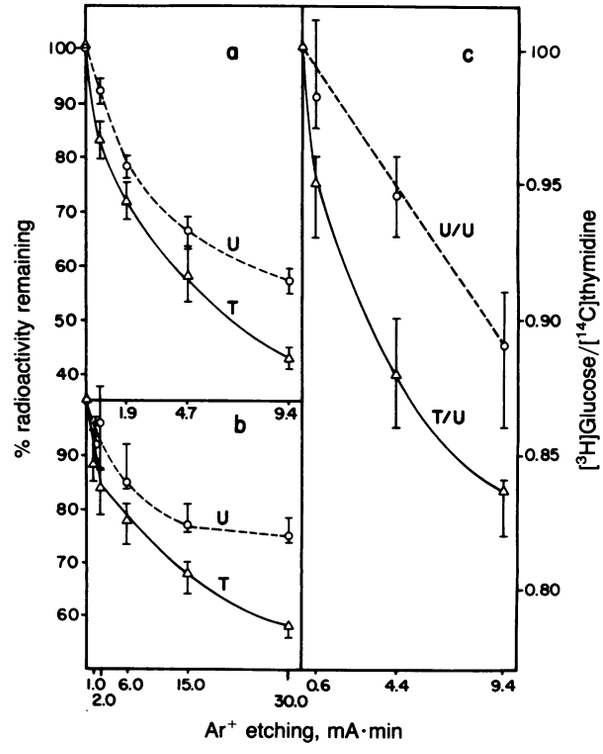

FIG. 3. Comparative etching rates of terminally (T) and uniformly (U) labeled DNA in phage T4 particles. Phage DNA was labeled with [^{3}H]glucose at hydroxymethylcytosine residues either uniformly over the entire length of the molecule (U, ○) or selectively in the last portion packaged (terminally labeled, T, △). In the experiment shown in *c* both types of [^{3}H]glucose labeling were combined with uniform labeling of DNA with [^{14}C]thymidine. Following Ar^+ etching for the indicated times (mA·min), radioactivity remaining on the support was determined by liquid scintillation spectroscopy and expressed as a percentage of the amount present originally (*a* and *b*) or as the ratio of ^{3}H:^{14}C label (*c*). Phage samples were prepared as follows: unfixed virus singly labeled with [^{3}H]glucose (*a*), glutaraldehyde-fixed virus singly labeled with [^{3}H]glucose (*b*), and glutaraldehyde-fixed virus doubly labeled with [^{14}C]thymidine (uniformly) and with [^{3}H]glucose either terminally (T/U) or uniformly (U/U) (*c*).

DNA packaged is more accessible to the Ar^+ beam because it is located at the periphery of the phage DNA mass.

DISCUSSION

The evidence presented in this paper strongly suggests that the first packaged T4 DNA is found toward the center of the capsid, whereas later packaged DNA surrounds it in the periphery. If DNA were condensed from outside to inside, we would expect shielding of the last packaged DNA, whereas we find the opposite result: this DNA is lost at up to twice the rate. This is the expected result for the outside DNA, given the average 50% distribution of label (8), on the basis of the concentric shell model for DNA arrangement (Fig. 4*b*); somewhat less than twice is predicted from our "spiral-fold" model (Fig. 4*a*). Since DNA may rearrange during packaging, the first DNA to enter the prohead would not necessarily be found in the center in early packaging intermediates. However, it should be noted that thin-sectioning studies of packaging intermediates *in vivo* frequently reveal incompletely filled heads, with DNA apparently concentrated toward the center of the prohead (13, 14). Such images might indeed follow rearrangement of the unfixed DNA (3). The fully packaged DNA in the intact virus we have employed for ion etching should not be capable of significant rearrangement. In fact, a condensation direction opposite to that we have determined (inside to outside) has generally been predicted, largely on the basis of the concentric shell model for packaged DNA (3). It was determined previously that the first T4 DNA end to be packaged was the first to be withdrawn from the phage head as well as suggested that the first end was deposited toward the center of the capsid (8). Were the concentric shell model correct, the etching results reported here would provide strong support for that determination of the order of entry and ejection of the T4 DNA ends. According to that model, if the first end condenses in the center, it should also be ejected first (3).

We share the view that the many common features of DNA packaging mechanism and packaged structure among the bacteriophage suggest a unitary basic arrangement (3). It also is probable that this structure must be highly ordered and invariant among particles—e.g., in phage T4, which does not have unique ends, DNA length is apparently controlled to about 1% by the constancy of this packaged configuration alone (15). In our view the principal observations among a number of bacteriophage relevant to a general structure are the following. (*i*) Packaged DNA can be cross-linked to the capsid along its entire length [λ (16)]. (*ii*) The first DNA to enter the head is found condensed in the center of the capsid [T4 (this work)]. (*iii*) Either the first DNA end to enter the head can be the last ejected [λ, φ29 (2, 17)] or the first DNA end to enter can be the first ejected [T4 (8)]. In keeping with the view that either directionality of ejection can operate among the phages, both DNA ends apparently lie near each other and near the DNA exit (and entrance) vertex of the full head [P2, T4 (8, 18)]. (*iv*) X-ray diffraction studies indicate an overall 25-Å parallel spacing of duplex DNA, which can, however, shift to generally looser packing in the event of deletions in the DNA [T4, P22, λ (5, 12)]. (*v*) DNA suddenly released from phage heads can maintain a ball-like structure (4). (*vi*) In both giant (elongated) phage T4 heads (12) and normal T4 heads observed by cryomicroscopy (19), there is an apparent arrangement of DNA parallel to the long axis of the head. (*vii*) Radial loops of varying length (rosettes) are the most frequently observed structures in DNA spread around disrupted capsids (8, 20, 21).

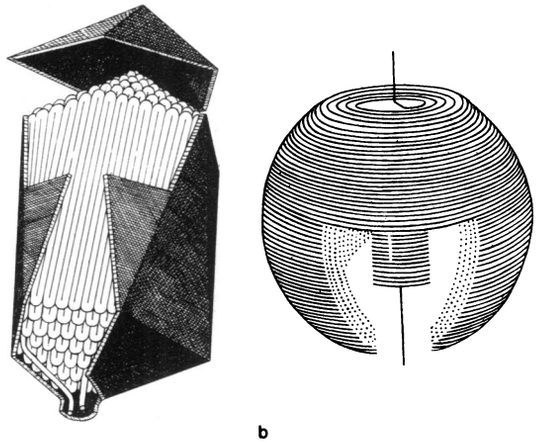

FIG. 4. Models for the arrangement of DNA inside the head of phage T4. (*a*) Spiral-fold model. (*b*) Concentric shell model (5, 12).

Assuming that there exists a single and basically invariant organization of condensed DNA among the bacteriophage, then several of the observations listed above are in conflict with the concentric shell model of phage DNA as originally proposed—specifically, *i, ii, iii,* and *vii*. The apparent contradiction between the cross-linking observation (*i* above) and the concentric shell model has been argued to be reconcilable by a modification that supposes that concentric loops can be laid down alternately in the center of the head and along the periphery so that there is no overall condensation direction, and the detailed packaging arrangement is variable among individual bacteriophage particles (22). We do not favor this view because it is in conflict with our observations reported here (*ii*). In addition, if DNA is wound up in concentric shells with gentle curvature, then it is not apparent why it should first make some tight windings. It is also not obvious that DNA possesses sufficient flexibility to be packaged in injectable form in such a randomized version of the concentric shell model.

We suggest the spiral-fold model shown in Fig. 4*a*. It is based upon the assumption that observations *i–vii* above are correct and that a single invariant structure applies among all of the bacteriophage particles. The most basic feature of this model is that DNA is supposed not to make gentle curves but is packed in sharply bent rods whose ends are in contact with the hemispherical caps. These folds of DNA are arranged radially about the long axis of the T4 head in spirally organized shells. The length of the folds, therefore, diminishes from the apices to the sides, with constant side-to-side spacing of the spirally organized shells. From the dimensions of the T4 head (23), the T4 DNA would fold into about 700–800 segments arranged in 15 or 16 shells, assuming overall 25-Å spacing of the shells. The length of the rods would range from 1050 Å to 533 Å, or about 300 base pairs to 160 base pairs of B-form DNA. There would necessarily be a sharp kink in DNA at the ends and a discontinuity in B-form DNA. Such a kink could result from a break in stacking of the bases, without loss of base pairing (24). In this proposed structure, DNA kinks could be separated by as few as 3 base pairs, allowing for sharp, 180° turns (24). Whether DNA at the ends of the folds were arranged in this or another (e.g., single-stranded) structure, DNA along the sides of the capsid and in contact with the caps would be structurally different, with possible differences in deformability at the ends and sides leading to the "Coke-bottle" shape sometimes visualized in the dried DNA mass (Fig. 1*a*). In addition, we also note a very recent report of 1–2% high-affinity sites for ethidium bromide binding in packaged phage DNA, consistent with kinks (25). To be packaged in such folded rods, traveling kinks in the DNA would necessarily be propagated along the entering DNA until contacts were made at the caps.

The spiral-fold model is in good overall agreement with the basic observations on packaged DNA arrangement listed above. (*i*) Without variability of packaged structure or packaging mechanism, contact is made throughout the packaged DNA length with the capsid surface. Probable differences in chemical reactivity as well as contacts between kinked DNA contacting the hemispherical caps, on the one hand, and duplex DNA along the sides of the capsid, on the other, would make uncertain any prediction of relative cross-linking frequencies expected along the DNA, and, of course, at the electron microscopic level, no regularity in cross-linking of capsid to DNA would be expected. (*ii*) The first packaged DNA can arrive in the center of the head without making tighter, thermodynamically unfavorable first windings, and DNA density is expected to be as high at the center as the periphery [unlike the concentric shell model (5)]. (*iii*) It appears equally probable, given this structure, that either DNA end could be withdrawn first, depending upon the particular phage, unlike the concentric shell structure (3). (*vi*) The parallel arrangement of duplexes along the long axis of the giant or normal T4 phage head is, of course, expected. (*vii*) The rosettes or radial loops most commonly observed around suddenly decondensed DNA in phage heads arise in a very simple way by withdrawing variable numbers of the folds of which such a structure is formed but are obscurely derived from the concentric shells. (*iv*) General duplex spacing of *ca.* 25 Å could arise due to DNA–DNA repulsions of the folds in shells of this model (Fig. 4*a*); the shells containing folds with sharp bends would appear to be as able to undergo continuous reorganizaion during packaging as in the concentric shell model, so that this spacing between the shells could increase in the event of deletions in the DNA. (*v*) In the spiral-fold model the condensate might well also give the overall impression of DNA curvature where individual duplexes are not clearly resolved (4). In fact, an artificial, kinked DNA condensate containing single-stranded nuclease-sensitive DNA folds (leading to a 200 to 400 base-pair limit DNA digest) can indeed result in a ball-like structure with DNA curvature comparable to that observed in disrupted phage condensates (ref. 26, figure 1*g*; ref. 4). On balance, therefore, there now appears to be generally better agreement between the spiral-fold model and most of the basic observations on packaged DNA structure than there is with the concentric shell model. The spiral-fold model, if correct, could have some interesting biological implications for the mechanism of DNA packaging. For example, packaging could be apparently kinetically discontinuous (17) because folds of DNA interact discontinuously with the capsid. Energy derived from straightening of kinks could be involved in injection of DNA into host cells. In any event, further experimental tests of the spiral-fold model and the concentric shell model for packaged DNA should be possible.

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1. Bricogne, G., Olson, A. J., & Harrison, S. C. (1983) *J. Mol. Biol.* **171**, 61–93.
2. Murialdo, H. & Becker, A. (1978) *Microbiol. Rev.* **47**, 529–576.
3. Earnshaw, W. C. & Casjens, S. R. (1980) *Cell* **21**, 319–331.
4. Richards, K. E., Williams, R. C. & Calendar, R. (1973) *J. Mol. Biol.* **78**, 255–259.
5. Earnshaw, W. C. & Harrison, S. (1977) *Nature (London)* **268**, 598–602.
6. Newcomb, W. W., Boring, J. W. & Brown, J. C. (1984) *J. Virol.* **51**, 52–56.
7. Bendet, I. & Risk, N. (1976) *Biophys. J.* **16**, 357–365.
8. Black, L. W. & Silverman, D. J. (1978) *J. Virol.* **28**, 643–655.
9. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
10. Black, L. W. & Showe, M. K. (1983) in *Bacteriophage T4*, eds. Matthews, C. K., Kutter, E. M., Mosig, G. & Berget, P. B. (Am. Soc. Microbiol., Washington, DC) pp. 219–245.
11. Luftig, R. B., Wood, W. B. & Okinaka, R. (1971) *J. Mol. Biol.* **57**, 555–573.
12. Earnshaw, W. C., King, J., Harrison, S. C. & Eiserling, F. A. (1978) *Cell* **14**, 559–568.
13. Lenk, E., Casjens, S., Weeks, J. & King, J. (1976) *Virology* **68**, 182–199.
14. Zachary, A. L. & Black, L. W. (1981) *J. Mol. Biol.* **149**, 641–658.
15. Kim, J.-A. & Davidson, N. (1974) *Virology* **57**, 93–111.
16. Widom, J. & Baldwin, R. L. (1983) *J. Mol. Biol.* **171**, 419–437.
17. Bjornsti, M., Reilly, B. W. & Anderson, D. L. (1983) *J. Virol.* **45**, 383–396.
18. Liu, L. F., Perkocha, L., Calendar, R. & Wang, J. C. (1981) *Proc. Natl. Acad. Sci USA* **78**, 5498–5502.
19. Adrian, M., Dubochet, J., Lepault, J. & McDowall, A. W. (1984) *Nature (London)* **308**, 32–36.
20. Kleinschmidt, A. K., Lang, D., Jacherts, D. & Zahn, R. K. (1962) *Biochim. Biophys. Acta* **61**, 857–864.
21. Virrankoski-Castrodeza, V. & Parish, J. H. (1980) *Arch. Microbiol.* **126**, 277–283.
22. Harrison, S. C. (1983) *J. Mol. Biol.* **171**, 577–580.
23. Eiserling, F. A. (1983) in *Bacteriophage T4*, eds. Mathews, C. K., Kutter, E. M., Mosig, G. & Berget, P. B. (Am. Soc. Microbiol., Washington, DC), pp. 11–24.
24. Crick, F. & Klug, A. (1976) *Nature (London)* **255**, 530–533.
25. Griess, G. A., Serwer, P. & Horowitz, P. M. (1985) *Biophys. J.* **47**, 49 (abstr.).
26. Laemmli, U. K. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4288–4292.