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Organization of double-stranded DNA in bacteriophages: a study by cryo-electron microscopy of vitrified samples

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In this paper it is shown that conformation and packing of double-stranded DNA within the head of bacteriophages lambda and T4 can be assessed by cryo-electron microscopy of vitrified specimens. Electron diffraction patterns show that DNA within vitrified bacteriophages has a B conformation. Electron micrographs of vitrified bacteriophages show domains within the head formed by a ~2.5-nm striation and arising from the DNA packing. The number of differently oriented domains seen within a vitrified bacteriophage depends upon the geometry of the DNA container: the bacteriophage capsid. The packing of DNA within bacteriophages seems then to be governed by at least two phenomena. The first is the tendency of DNA to form local alignments (nematic liquid crystals). The second is the orientation of these liquid crystals by the bacteriophage capsid. From these observations we propose a possible packaging mechanism: constrained nematic crystallization.

Key words: cryo-electron microscopy/DNA packaging/lambda/liquid crystals/T4

Introduction

The genome of many bacteriophages is a double-stranded DNA molecule which is densely packed in a protein capsid; to infect the bacterial cell, it is linearly transferred through the cell wall (Goldberg, 1980). In order to be packed at the high density of 0.8 mg/ml observed in the head (Kellenberger *et al.*, 1986) and to make the transfer possible without forming knots, the DNA must be properly arranged. In spite of numerous studies, there is not as yet a consensus on the arrangement of the DNA in the bacteriophage head (Earnshaw and Harrison, 1977; Black *et al.*, 1985; Serwer, 1986). All investigations were indirect, because conventional electron microscopy was unable to depict the DNA in the head. The recent development of cryo-electron microscopy of vitrified specimens has changed the situation; not only can the arrangement of the DNA molecule be observed directly, but electron diffraction can be used to define the conformation and orientation of the DNA. In the present work, we have used this technique to study the arrangement of the DNA in the head of the bacteriophages lambda and T4 and some of their morphological variants. We find that the DNA arrangement is on the whole less orderly than previously believed and does not directly reflect the manner in which DNA has been packaged.

Results

Pure DNA

DNA molecules dissolved in 50 mM NaCl tend to orient in a parallel direction when a thin film of solution is partially dried before vitrification. By electron diffraction the specimen is characterized by two perpendicularly oriented reflections (Figure 1A). The first one corresponds to 0.33 ± 0.01 nm and arises from the base stacking distance in the B DNA. The second corresponds to the packing distance between adjacent filaments. It varies from 3.0 to 1.7 nm depending on the time the thin film is allowed to dry before vitrification. Higher orders of this reflection are not observed, indicating that the parallel arrangement of the molecules is not very regular. DNA molecules are also directly visible on micrographs as a faint striation (Figure 1B). The spacing varies within a specimen and with the amount of drying prior to vitrification. Lateral spacings in the range 2.0–4.5 nm are generally measured by optical diffraction. In all cases, the striation forms small (15–30 nm) domains with slightly differing orientations.

Giant T4 bacteriophages

In giant T4 bacteriophages, the arrangement of the DNA is well revealed by both electron diffraction and direct imaging. These particles, produced by some mutants in gene 23, have a much longer head than the wild-type and contain several T4 genomes (Doermann *et al.*, 1973). As is the case for DNA, they can be oriented in thin liquid film preparations. The electron diffraction of such a specimen is shown in Figure 2. The defocused diffraction pattern (insert), corresponding to a low magnification image, shows the parallel arrangement of particles and serves to orient the diffractogram in respect of the object. This figure shows that the 0.33-nm reflection of the stacked bases is meridional, implying that the main direction of the DNA is approximately parallel to the long head axis. In the equatorial direction, the arrangement of the molecules gives rise to a reflection corresponding to 2.3 ± 0.2 nm. Micrographs of the bacteriophage (Figure 3A–C) confirm the parallel arrangement of the DNA along the axis of the head with a spacing corresponding here to 2.4 ± 0.2 nm (optical diffractogram in Figure 3A). The exact pattern of this arrangement differs, however, from one particle to the other. In Figure 3B (enlarged view of 3A), the 2.4 nm striation is only visible in six or seven domains or bands which are each ~10 nm high. The bands are perpendicular to the head axis and separated by distances of 60–120 nm. In Figure 3C, the striation is visible in many domains of various sizes, irregularly distributed over the whole head. In most cases the striation becomes disoriented at both ends of the particle where it is frequently parallel to the surface of the capsid.

T3 and lambda bacteriophages

Electron diffraction patterns of concentrated T4 or lambda solutions show a ring corresponding to 0.33 nm and arising from the base stacking distance in B DNA (not shown). Direct imaging of these phages shows the packing of the DNA; it appears to be less regular than in the specimen described above. In general,

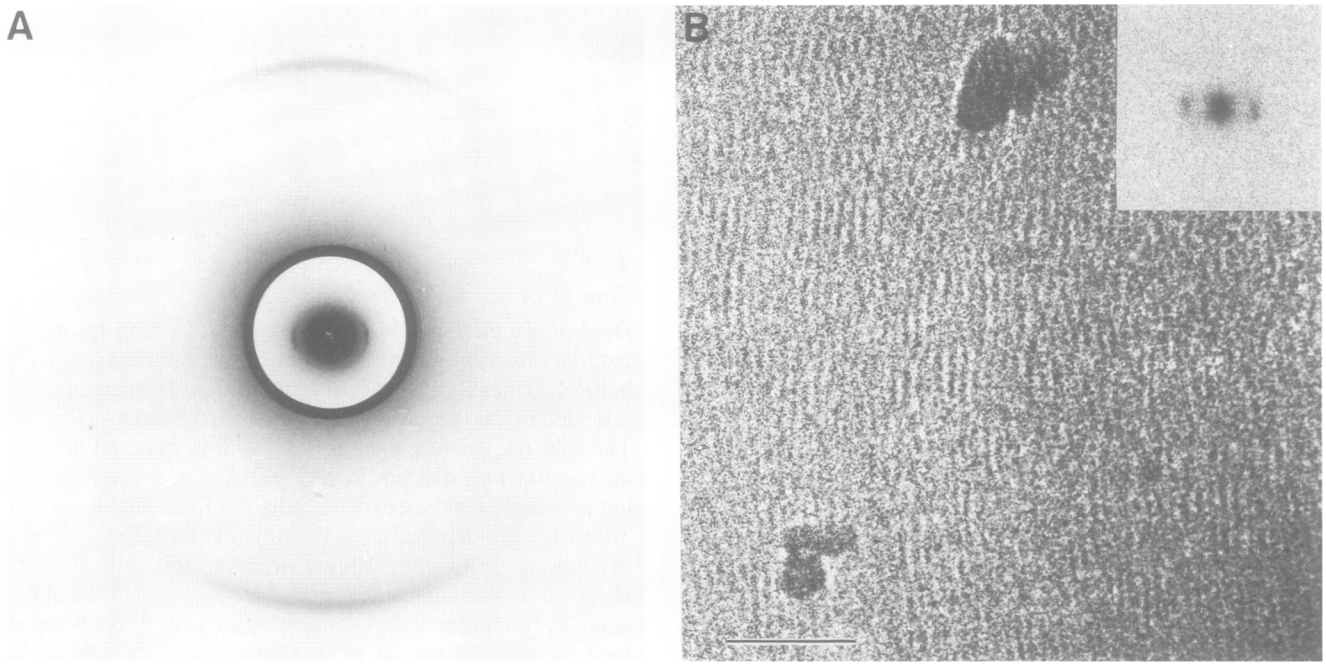

Fig. 1. A. Electron diffraction pattern of a vitrified DNA suspension. DNA molecules have been aligned by partial drying of the suspension before freezing as described in Materials and methods. The meridional arc is located at 0.33 nm^{-1}. Taking the well-characaterized rings of cubic ice (Dubochet *et al.*, 1982) as reference, the DNA meridional arc corresponds to 0.328 ± 0.007 nm. The central part is an underexposed part of the pattern so that the equatorial arc located at 2.8 nm^{-1} is visible. **B.** Electron micrograph of a vitrified DNA suspension. DNA molecules were aligned by partial drying. The striations are separated by 4.5 nm. Insert: optical diffraction pattern. The bar in all images corresponds to 50 nm.

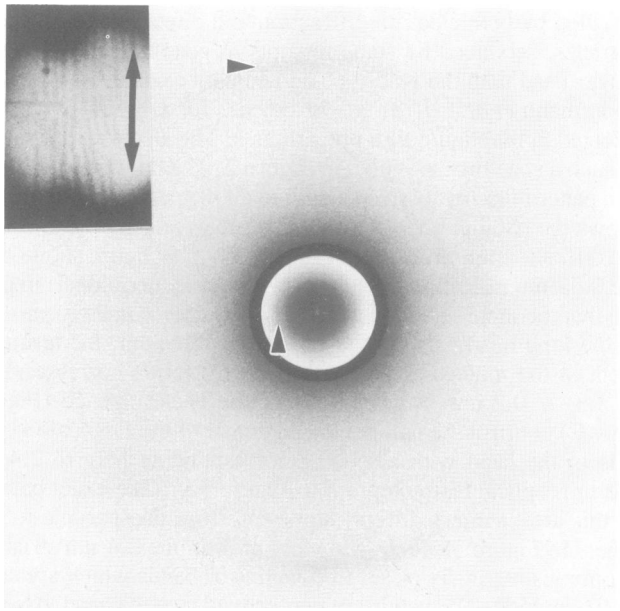

Fig. 2. Electron diffraction pattern of aligned giant T4 bacteriophages. The meridional [$(0.33\ nm)^{-1}$] and equatorial 2.2 nm^{-1} arcs are indicated by arrows. The central part is underexposed so that the weak equatorial arcs are detectable. Insert: same electron diffraction pattern as the one shown in the figure but with the diffraction lens largely underfocused. On the underfocused diffraction pattern, giant T4 bacteriophages can be recognized. The bacteriophage long axis is indicated by an arrow. As rotation between focused and underfocused pattern can be neglected, mean orientation of the DNA molecule can be oriented in respect of the bacteriophage long axis.

the DNA is arranged in domains characterized by parallel striations. The striations have a characteristic distance of ~2.5 nm. Examples are shown in Figure 4A, depicting a field of lambda bacteriophages and in Figure 4B, a T4 particle. Empty capsids of lambda (Figure 4A) do not show any internal structure, thus confirming that the striation observed in the other particles is due to the DNA. The same holds for empty capsids of T4 giants and of wild-type T4 (not shown). The striation due to the regular arrangement the DNA is never visible over the whole particle but is limited to several domains with various orientations. These domains also vary in size, although they are typically in the 20-nm range. In prolate head of wild-type T4, the striation is predominantly parallel to the long axis of the head. The optical diffractogram of single particles shows therefore a more or less uniform circle corresponding to about 2.5 nm for lambda (insert, Figure 4A). The optical diffraction pattern of T4 images shows a circle of the same diameter but with dominant intensity situated perpendicular to the long axis of the head (insert, Figure 4B).

Micrographs never show singularity at the centre of the particle, complete order over the whole particle, or a clear discontinuity as could be caused by an axis or a centre of symmtry. T4 phages observed in thin vitrified films, however, are not randomly oriented. Because the particles are elongated by their tails, most of them lie more or less flat in the vitrified film. The projection down the long axis of the particle is therefore not represented in the specimen. We obtained this view by observing T4-related particles produced by a mutant in gene 10, which show a normal mature head but no tail (Figure 4C). The DNA in this projection appears as a number of domains with differing orientations, confirming that the DNA packing is long range disordered.

Discussion

The organization of the DNA in the giant T4 closely resembles that of a gel of oriented B DNA, as becomes evident when comparing their electron diffractograms. The DNA is approximately parallel to the long axis of the head and is regularly packed

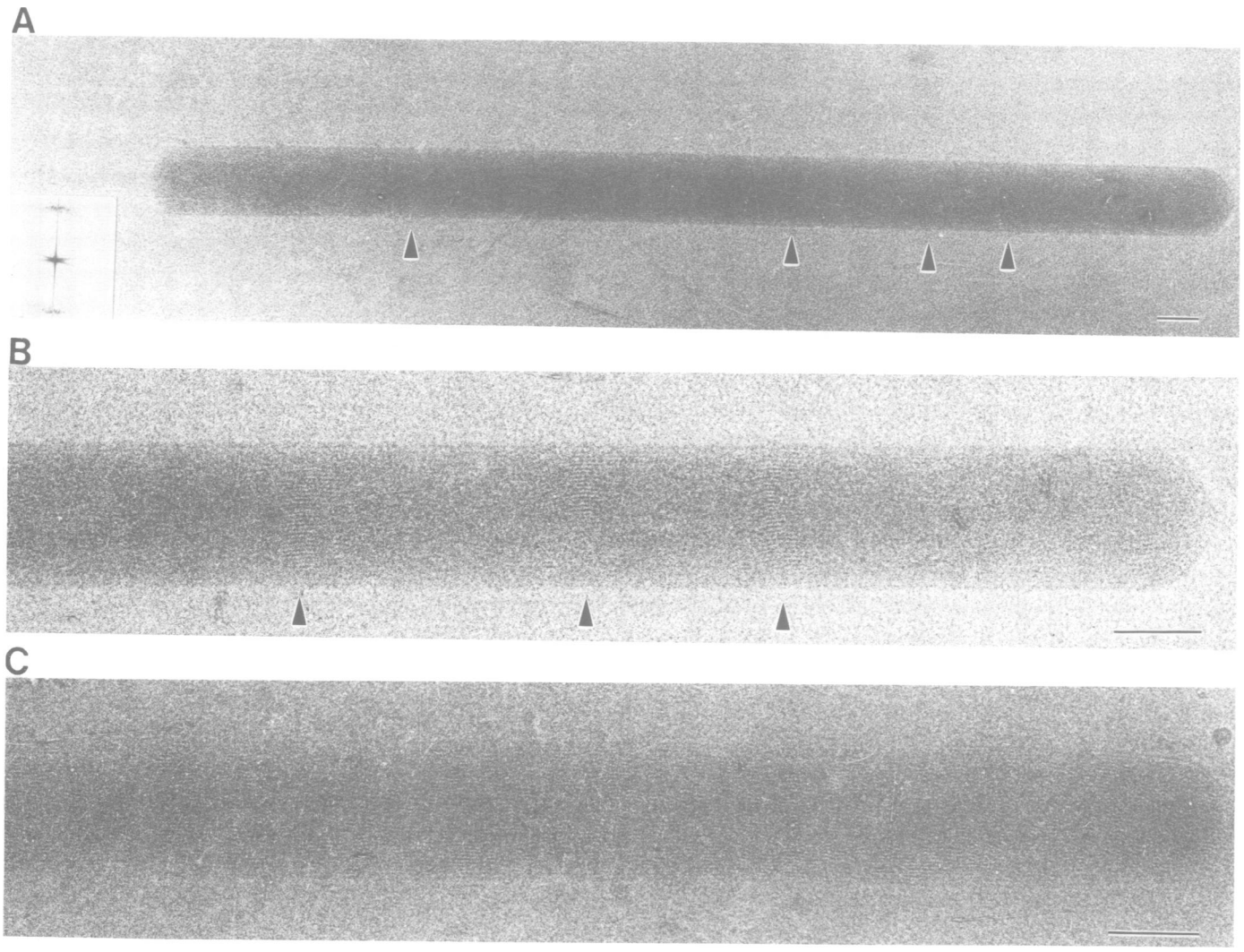

Fig. 3. A. Electron micrograph of a vitrified giant T4 bacteriophage. The arrow indicates the position of bands formed by 2.5-nm striations having a strong contrast. Insert: optical diffraction pattern of the bacteriophage head. **B.** Enlargement of **A**. **C.** Electron micrograph of a vitrified giant T4 bacteriophage. The 2.5nm striation is present over the entire head length.

perpendicular to this direction. Assuming the DNA segments to be hexagonally packed, their centre to centre distance is about 2.8 nm. The DNA does not form a perfectly ordered crystal, however, as can be judged from the absence of higher order equatorial reflections. Images and electron diffraction patterns are typical for nematic liquid crystalline phases (Vainshtein, 1966). A better characterization of the long range organization can be deduced from direct images. The striation seen in the giant heads is too pronounced to reflect a single layer of DNA molecules. To explain the contrast, a number of molecules must be superimposed and therefore aligned along the viewing direction. It is also observed that the contrast striation is regular over domains of some tens of nm. In these domains the DNA seems to be arranged regularly in the plane perpendicular to the DNA fibres. The organization of the DNA between the domains is not visible but this does not necessarily imply that there is no periodic order there. It could just reflect an unfavourable orientation of the alignments. The following continuous helical model can account for the repeated domains of striation observed along the length of the giant shown in Figure 3A,B. The model assumes that DNA segments are packed in a hexagonal lattice perpendicular to the long axis of the head and that the resulting bundle of filaments is twisted into a super helix. The striation seen in the image corresponds to regions where rows of filaments are aligned with the viewing direction. The distance between striated domains corresponds thus to a 60° rotation of the helix. By consequence, the pitch of the helix would then be ~360 nm and the pitch angle would vary from 90° in the centre of the particle to about 70° at the outer circumference. This model is similar to one proposed by Earnshaw *et al.* (1978).

Observations of DNA packing in the giants do not support a completely ordered model. The distances between the striated domains in Figure 3A,B are not regular, reflecting a variation of the helix parameters of our model. In other micrographs, the distribution of the domains is even less regular, differing in shape and size. Figure 3C is a typical example: the numerous striated domains seem to be located randomly throughout the particle. Regions where the DNA seems to be regularly packed alternate with regions where the bundle is twisted or disordered. Furthermore, at both ends of the particle, the alignment of the DNA is no longer parallel to the long axis of the head.

The DNA in bacteriophage lambda is in the B form, as observed previously by X-ray diffraction (Earnshaw and Harrison, 1977; Earnshaw *et al.*, 1978), and is arranged into a number

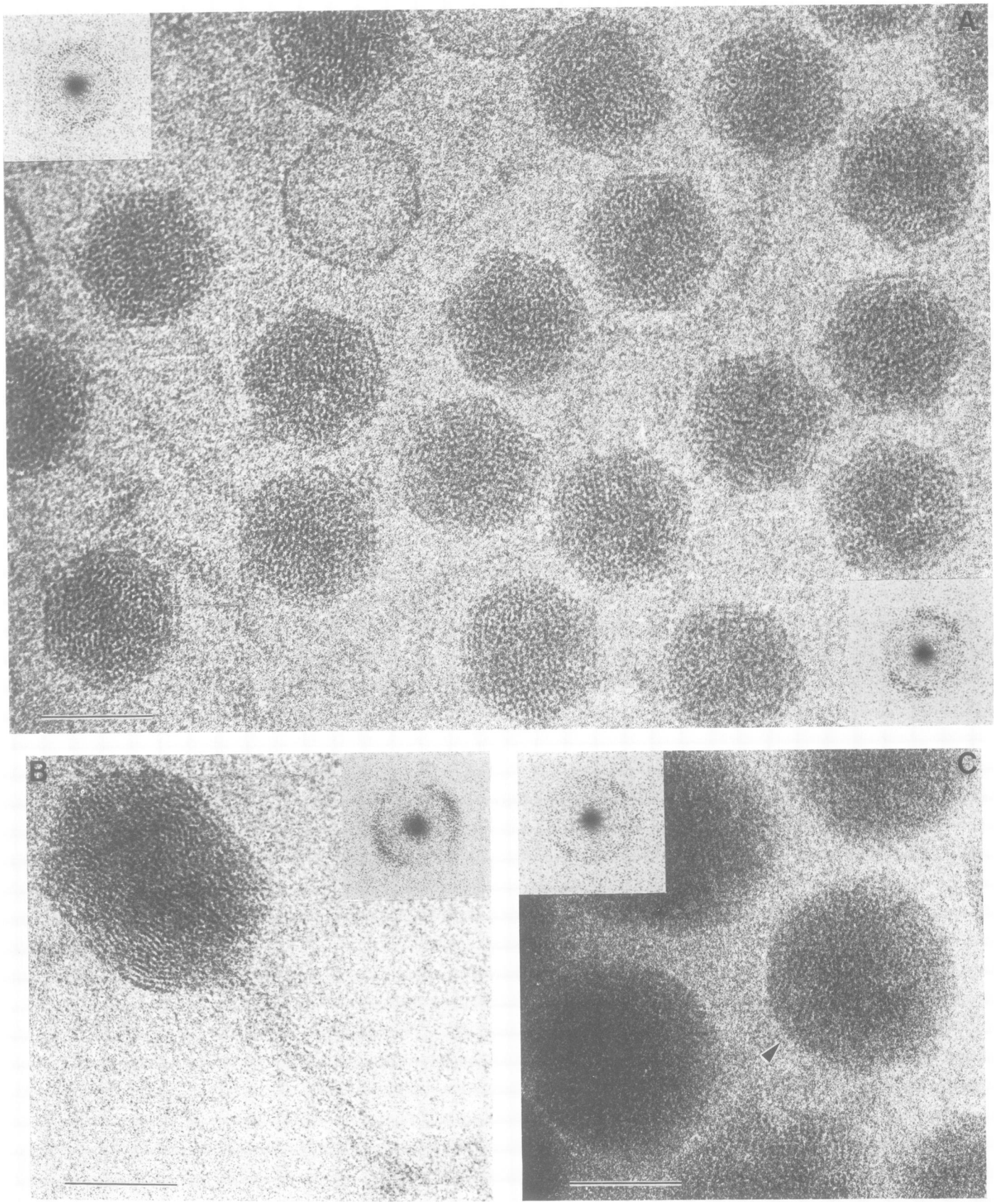

Fig. 4. A. Electron micrograph of vitrified suspension of lambda bacteriophages. Note that empty bacteriophages do not show any structure in their head: Insert: optical diffraction patterns of a single bacteriophage head showing a ring-like intensity distribution located at 2.4 nm^{-1}. **B.** Electron micrograph of a bacteriophage T4 embedded in vitreous ice. Insert: optical diffraction pattern of the bacteriophage head. **C.** Electron micrograph of 10^- heads. The relative isotropy of 10^- heads compared with wild-type bacteriophages enable particles to be obtained that are 'randomly' oriented in the ice film. the arrows indicate end-on views of 10^- heads. Insert: optical diffraction pattern of a single particle end-on view. Note the circular distribution of intensities.

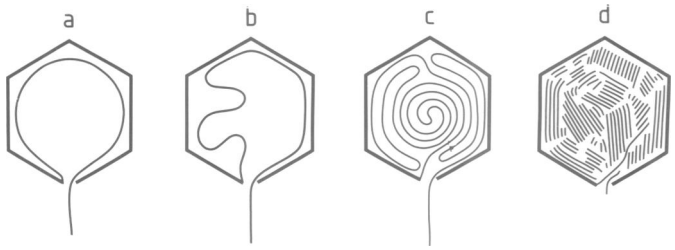

Fig. 5. Schematic representation of DNA packaging by constrained nematic crystallization: **a**–**d** different states of packing. When DNA enters the capsid **(a)**, it bends due to the finite size of the capsid **(b)**. While more DNA enters it rearranges continuously keeping the whole volume continuously filled **(c)**. While most of the DNA segments are oriented by the capsid surface, they are forced closer together. To accommodate the increasing packing density, DNA segments become more and more ordered forming liquid crystalline domains **(d)**. In **(d)**, the DNA segments are not connected. This deliberate omission is due to the absence of experimental data concerning the connection between the segments forming liquid crystalline domains. It does not change the significance of our packaging model. In **a**–**d** we also supposed that one end of the DNA molecule is not free to move. This is to prevent the formation of knots during the rearrangements undergone by the DNA molecule during packaging.

of domains of regularly packed parallel segments. Contrary to what happens in the main part of the giant, the domains seem to be randomly oriented one in respect to the other. Neither a preferential orientation nor symmetry is observed. The DNA arrangement in wild-type T4 appears to be an intermediate case between those existing in giant T4 and lambda. The domains may have an orientation, although that of the long axis of the head is favoured. However, both side and end-on views of T4 bacteriophage show that the domain, where DNA segments are parallel to the long axis of the head, is long-range disordered.

In isometric bacteriophage heads (Figure 4A), DNA forms liquid crystalline domains having varying orientations. The more the head is prolate (Figures 3C and 4B), the more the DNA segments are oriented in the direction of the long axis of the particle. In the terminal part of these particles, DNA segments parallel to the capsid surface are often observed (Figures 3C and 4B). These observations allow us to conclude that DNA packing in bacteriophages is governed by at least two phenomena. The first is the tendency of DNA to form nematic liquid crystals. The second is the orientation of these crystals by the surface of the DNA container: the bacteriophage capsid. The first phenomenon is responsible for the short-range order of the structures observed in bacteriophage heads, while the second governs the long-range order of these structures. As a consequence of these two phenomena, the structure of DNA in bacteriophages does not directly reflect the mechanism of packaging.

The data presented here do not allow the exact path of the long DNA molecule in the phage head to be followed. It is not even possible to deduce how the various ordered domains are connected. One notes, however, that the DNA is very densely packed in domains and this high density could not be obtained if the DNA segments were disordered. Furthermore, micrographs show that the density of DNA is constant throughout the bacteriophage head. Consequently, the connection between the domains must be either relatively ordered or limited to very short regions. The latter would require that the DNA can form local bends. The fact that DNA in suspension forms ordered, differently oriented domains of straight DNA segments (Figure 1B) of a dimension smaller than the length of the DNA molecule, provides evidence that DNA molecules at high concentrations are locally bent.

Two major structural models for the arrangement of the DNA in the bacteriophage head have been proposed from previous studies by X-ray diffraction, electron microscopy and ion etching (North and Rich, 1961; Earnshaw *et al.*, 1976, 1978; Earnshaw and Harrison, 1977; Richards *et al.*, 1972; Black *et al.*, 1985). They offer suggestions on the mechanism allowing the reversible packing of the long DNA molecule in the small head volume. The spooling model (Richards *et al.*, 1973; Earnshaw and Harrison, 1977) states that the DNA is continuously wound around a predetermined axis. This implies that a discontinuity exists along the spool axis and that there is some kind of rotational symmetry around it. Such features are not observed in our images of the lambda and T4 bacteriophages. The 'spiral fold' model (Black *et al.*, 1985) proposes that the bacteriophage head is filled with closely packed straight fragments of DNA, linked by short kinks. This model results in a nearly crystalline arrangement filling the whole phage head. This feature also is not observed in images of vitrified specimens of wild-type particles. The spiral fold model is, however, easily reconciled with the data presented here, if it is accepted that the folded fragments are packed into several domains with differing orientations. The spooling model would also be reconciled with our data by introducing long-range disorder in the position and direction of spool portions. We feel, however, that the observations presented here are accounted for, and the functions of the phage head explained in a straightforward manner by still another model in which the DNA structure is supposed to be flexible enough to bend in order to fit its container, but rigid enough to form nematic liquid crystals.

The model of DNA packing which we propose is illustrated in Figure 5 for lambda bacteriophage. When entering the phage head, the DNA occupies the whole volume at its disposal (a). This does not need to be the whole capsid volume; it could also be, for example, the volume left as a protein scaffold is progressively removed. While more DNA enters, it rearranges continuously, keeping the whole volume uniformly filled (b). The finite size of the capsid forces the DNA to bend. DNA segments are thus forced closer together (c) and oriented by the capsid surface. In order to accommodate the increasing packing density, they become more and more ordered (d). The rearrangement of the DNA continues until the whole genome is packaged. The model also applies to wild-type T4 and giants but, in these cases, the long-shaped head defines a preferential direction for the DNA packing.

In the drawing of Figure 5d, the connections between the DNA segments forming ordered domains were omitted. Depending upon the way the segments are connected DNA is bent more or less sharply. Taking into account the theoretical complexity represented by DNA bending (Crick and Klug, 1975), as well as the absence of experimental data, we do not represent any bent DNA segments in Figure 5d. The absence of any data concerning the connections between the segments forming the ordered domains does not change the significance of our packaging model. A basic requirement of our model is that DNA molecules are very free to move along and around each other. This hypothesis is supported by theory and experiments about densely charged polymers in ionic solution (Record *et al.*, 1978; Manning, 1978). It is shown that in a wide range of concentration around physiological values, counter ions are delocalized along the DNA molecule. The fact that concentrated aqueous solutions of DNA tend to form liquid crystals instead of real crystals points in the same direction (Livolant, 1984; Rill, 1986). It should be noted that the local packing of DNA in bacteriophage is similar to that

adopted by DNA in solution at a similar concentration (Rill, 1986). The observation that in lambda mutants containing only a fraction of the wild-type genome, the DNA is packed more loosely (Earnshaw and Harrison, 1977), provides further evidence that the arrangement is continuously readjusted during head filling. The observation that upon osmotic shock the expelled genome of lambda or T4 seems to be arranged in concentric circles (Richards *et al.*, 1973) is understood, in terms of the packaging model, to be the result of the rearrangement taking place when the constraints of the capsid containment are removed. Our model of constrained nematic crystallization accounts for the data presented in this paper but also for those obtained by X-ray diffraction (Earnshaw *et al.*, 1978), crosslinking experiments (Haas *et al.*, 1982; Widom and Baldwin, 1983) and flow dichroism (Gellert and Davis, 1964; Hall and Schellman, 1982). The injection of the DNA into bacteria could simply correspond to the reverse of the filling of the head. This holds when the end which last enters the capsid is injected first (Black and Silverman, 1978). It is more difficult to imagine a simple ejection process if, as reported for lambda (Emmons, 1974), the DNA end which first enters the capsid is also the one which leaves it first. However, the rearrangements of the DNA structure necessary to reverse the direction of injection compared with that of packaging are conceivable if we suppose, as in our model, that DNA segments are free to move in respect of each other.

Materials and methods

Preparation of bacteriophages

Bacteriophage T_4D^+ wild-type was prepared as described by Kellenberger (1968) and further purified through a sucrose gradient. T_4 giants were isolated from *Escherichia coli* CR infected with bacteriophage $T_4$23ptg19-80, a mutant which produces in addition to normal phages a few percent of giant and isometric particles. They were concentrated and purified on a $CsCl_2$ gradient (Doermann *et al.*, 1973). Bacteriophage λ were produced by using the mutant λ CI-.S am7 in *E. coli* K12-W3110 (Arber *et al.*, 1983). The mutant was chosen because it does not lysogenize *E. coli*. Bacteriophages were concentrated from the crude lysate by precipitation with PEG-6000 (Yamamoto *et al.*, 1970) and further purified through a sucrose gradient.

All bacteriophages were stored at 4°C, non-frozen, in 60 mM phosphate buffer pH 7 (T_4 and T_4 giants) or 10 mM Tris-buffer pH 7 (λ) respectively, both buffers containing 1 mM $MgSO_4$ and 0.05% NaN_3. From high concentration gradients phages were transferred into buffer by dialysis against gradually decreasing osmolarity maintained by sucrose or NaCl respectively.

Electron microscopy

All specimens except those aligned were prepared as described by Adrian *et al.* (1984)). A droplet of the suspension was deposited on a perforated carbon film treated by glow discharge in air. The grid, mounted on a guillotine-like frame, was partially dried with filter paper, and the guillotine released immediately, plunging the grid into a cryostat of liquid ethane. Similar results, as far as the bacteriophage structure is concerned, were obtained whether or not the perforated carbon film was coated with an additional carbon film. Alignment of particles was achieved using the procedure referred to as the 'bare grid method' (Adrian *et al.*, 1984) but using grids covered with perforated carbon film. The suspension was deposited on the grid which was then mounted on the guillotine. The suspension layer was left to evaporate while being observed under a low-power microscope. The guillotine is released when most of the grid squares are filled by a layer having the right thickness. The evaporation time depends upon the initial thickness of the suspension layer deposited onto the grid. This was generally equal to 10–30 s. It was found that the preparation of the suspension film was easier if the grid was washed before the deposition of the suspension with a solution of 1% Triton X-100. Besides the reproducibility of the film suspension, no difference was observed whether or not the grid was washed with Triton X-100.

The electron microscope used in this study was a Philips EM400 operated at 80 kV. The microscope was fitted with an improved blade-type anticontamination device (Homo *et al.*, 1984). Frozen specimens were inserted into the cryo-specimen holder (Philips PW6591-100) under liquid nitrogen, and rapidly inserted into the electron micrscope.

Electron diffraction patterns and images were recorded on Kodak electron image film SO-163 which was developed for 12 min in D-19 developer at full strength. Images were recorded at 43 000× magnification using the low-dose unit of the microscope. The electron dose recorded on the film was such that its average density was ~1.0; the specimen was then irradiated by about 1000 e^-/nm^2.

Unstained vitrified objects are mainly imaged by phase contrast (Lepault and Pitt, 1984). Micrographs were then focused so that the phase contrast transfer function was maximum at a spatial frequency of 2.5 nm^{-1}. This is achieved when the image is defocused by 0.7 μm. Under these conditions, the bacteriophage tails do not display contrasted features (Lepault and Leonard, 1985).

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References

Adrian,M., Dubochet,J., Lepault,J. and McDowall,A.W. (1984) *Nature*, **308**, 32–36.

Arber,W., Esquist,L., Hohn,B., Murray,N.E. and Murray,K. (1983) in Hendrix, R.W., Roberts,J.W., Stahl,F.W. and Weisberg,R.A. (eds), *Lambda II*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 433–466.

Black,L.W. and Silverman,J. (1978) *J. Virol.*, **28**, 643–655.

Black,L.W., Newcomb,W.W., Boring,J.W. and Brown,J.C. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 7960–7964.

Crick,F.H. and Klug,A. (1975) *Nature*, **255**, 530–533.

Doermann,A.H., Eiserling,F.A. and Boehner,L. (1973) *J. Virol.*, **12**, 374–385.

Dubochet,J., Lepault,J., Freeman,R., Berriman,J.A. and Homo,J.-C. (1982) *J. Microsc. (Oxford)*, **128**, 219–237.

Earnshaw,W.C. and Harrison,S.C. (1977) *Nature*, **268**, 598–602.

Earnshaw,W.C., Casjens,S. and Harrison,S.C. (1976) *J. Mol. Biol.*, **104**, 387–410.

Earnshaw,W.C., King,J., Harrison,S.C. and Eiserling,F.A. (1978) *Cell*, **14**, 559–568.

Emmons,S. (1974) *J. Mol. Biol.*, **83**, 511–525.

Gellert,M. and Davies,D.R. (1964) *J. Mol. Biol.*, **3**, 341–347.

Goldberg,E.B. (1980) In Randall,L.I. and Philipson,L. (eds), *Receptors and Recognition*. Series B, Vol. 7.

Haas,R., Murphy,R.F. and Cantor,C.R. (1982) *J. Mol. Biol.*, **159**, 71–92.

Hall,S.B. and Schellman,J.A. (1982) *Biopolymers*, **21**, 1991–2010.

Homo,J.-C., Booy,F., Labouesse,P., Lepault,J. and Dubochet,J. (1984) *J. Microsc. (Oxford)*, **136**, 337–340.

Kellenberger,E. (1968) *Virology*, **34**, 549–561.

Kellenberger,E., Carlemalm,E., Séchaud,J., Ryter,A. and de Haller,G. (1986) In Gualerzi,C.O. and Pon,C.L. (eds), *Bacterial Chromatin*. Springer Verlag, Berlin and Heidelberg, pp. 11–25.

Lepault,J. and Leonard,K. (1985) *J. Mol. Biol.*, **182**, 431–441.

Lepault,J. and Pitt,T. (1984) *EMBO J.*, **3**, 101–105.

Livolant,F. (1984) *Eur. J. Cell Biol.*, **33**, 300–311.

Manning,G.S. (1978) *Q. Rev. Biophys.*, **11**, 179–247.

North,A.C. and Rich,A. (1961) *Nature*, **191**, 1242–1245.

Record,M.T., Anderson,C.F. and Lohman,T.M. (1978) *Q. Rev. Biophys.*, **11**, 103–178.

Richards,K.E., Williams,R.C. and Calendar,R. (1973) *J. Mol. Biol.*, **78**, 255–259.

Rill,R.L. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 342–346.

Serwer,P. (1986) *J. Mol. Biol.*, **190**, 509–512.

Vainshtein,B.K. (ed.) (1966) *Diffraction of X-rays by Chain Molecules*. Elsevier, Amsterdam.

Widom,J. and Baldwin,R. (1983) *J. Mol. Biol.*, **171**, 419–437.

Yamamoto,K.R., Alberts,B.M., Benzinger,R., Lawhorne,L. and Treiber,G. (1970) *Virology*, **40**, 734–744.

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