Double-Stranded DNA Organization in Bacteriophage Heads: An Alternative Toroid-Based Model

Nicholas V. Hud
Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, California 94551 USA

ABSTRACT Studies of the organization of double-stranded DNA within bacteriophage heads during the past four decades have produced a wealth of data. However, despite the presentation of numerous models, the true organization of DNA within phage heads remains unresolved. The observations of toroidal DNA structures in electron micrographs of phage lysates have long been cited as support for the organization of DNA in a spool-like fashion. This particular model, like all other models, has not been found to be consistent will all available data. Recently we proposed that DNA within toroidal condensates produced in vitro is organized in a manner significantly different from that suggested by the spool model. This new toroid model has allowed the development of an alternative model for DNA organization within bacteriophage heads that is consistent with a wide range of biophysical data. Here we propose that bacteriophage DNA is packaged in a toroid that is folded into a highly compact structure.

INTRODUCTION

Despite considerable effort, the question of how double-stranded DNA is organized within bacteriophages such as T1, T2, T3, T4, P2, P22, ϕ29, and λ remains unresolved. Various biophysical investigations have generated a series of models that include DNA being organized like a ball of string (Richards et al., 1973), as thread on a spool (Klimenko et al., 1967; Richards et al., 1973; Earnshaw and Harrison, 1977), in a unidirectional array of linear segments (Black et al., 1985), and as nematic liquid crystals (Lepault et al., 1987). However, not one of these models is consistent with all presently available data.

Toroidal DNA condensates have been observed repeatedly in electron micrographs of gently lysed phages (Klimenko et al., 1967; Richards et al., 1973; Earnshaw et al., 1978) and thus represent a distinct possibility for the structure of condensed DNA within phage heads. Furthermore, the formation of toroidal structures in vitro by the complexing of DNA with viral DNA-condensing agents has made the prospect of a toroidal morphology for DNA organization attractive in terms of viral self-assembly (Gosule and Schellman, 1976). These observations have prompted several investigators to model the DNA inside of bacteriophage heads into spool-like structures, paying considerable attention to the details of spool orientation and the mode of DNA wrapping in attempts to reconcile experimental data with a toroidal structure (Earnshaw and Harrison, 1977; Earnshaw et al., 1978; Kosturko et al., 1979; Harrison, 1983). However, despite the initial acceptance of toroid-based organization by many, additional studies that were found to be inconsistent with the spool model (not with the existence of toroids per se) have led to the development of several different models (Haas et al., 1982; Widom and Baldwin, 1983; Black et al., 1985; Welsh and Cantor, 1987).

Some investigators (Tikchonenko, 1975; Lepault et al., 1987) have expressed the opinion that although they may be related to the organization of DNA within intact phage heads, the presence of toroids in phage lysates is most likely the result of DNA rearrangement after its expulsion from phage heads. However, this presumed rearrangement has never been proven. On the basis of our studies of DNA toroid formation in vitro (Hud et al., 1995), we believe that DNA condensed in a manner significantly different from the toroid morphology would first have to pass through a state of complete disassociation before it could rearrange into the toroidal form. Such a process seems an unlikely source of the toroids observed in phage lysates, for in these preparations toroids are frequently found in close proximity to phage heads, from which they seem to have been released (Klimenko et al., 1967; Richards et al., 1973; Earnshaw et al., 1978). Here we present an alternative toroid-based model for the organization of double-stranded DNA within bacteriophage heads. This model is shown to be consistent with a wide range of data and is in many ways reminiscent of several previous models for bacteriophage DNA packaging, models that among themselves may seem to be incompatible.

DEVELOPMENT OF AN ALTERNATIVE TOROID-BASED MODEL

Richards et al. (1973) reported that toroids expelled from the bacteriophage T7 typically measure 800 Å in outside diameter with a 300 Å diameter hole. Klimenko et al. (1967), on the other hand, reported a rather wide distribution of dimensions for toroids in the lysates of T2. If the genomes of these bacteriophages are in fact packaged within toroidal structures, then each toroid for a particular

Received for publication 15 May 1995 and in final form 17 July 1995.
Address reprint requests to Dr. Nicholas Vincent Hud, Department of Chemistry and Biochemistry, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90095. E-mail: hud@uclave.mbi.ucla.edu.

0006-3495/95/10/1355/08 $2.00

type of phage should be of equal volume and of similar dimensions, an assertion that is inconsistent with the range of measurements reported for T2 toroids. Electron micrographs presented by Klimenko et al. (1967), presumably representative of those from which measurements were taken, reveal a considerable amount of DNA extraneous to toroids. These micrographs suggest that the reported distribution of T2 toroid sizes may actually be a result of partial toroid decondensation after their expulsion from phage heads. If we consider bacteriophage toroids as having a circular cross section, an approximation supported by previous investigations of toroidal DNA condensates (Arscott et al., 1990), then a toroid volume calculated from the mean value of dimensions reported for T2 toroids is only 50% of the expected volume for a fully condensed T2 genome (Tikchonenko, 1975), whereas the volume of a T2 toroid that lies at the upper end of the reported dimensions is large enough to account for 96% of the expected volume of a fully condensed T2 genome. Thus we believe that the most probable dimensions for a toroid inside a T2 head are those of the largest volume toroid observed by Klimenko et al. (1967), namely 1300 Å in outside diameter with a 500 Å diameter hole. In the case of T7, the single values reported for toroid outside diameter and toriod hole diameter seem to be quite accurate, for the toroid volume based on these dimensions is 94% of that expected.

The icosahedral head of T2 is prolate with a major axis of ~1160 Å and a minor axis of ~800 Å, whereas the head of T7 is more isometric with a maximum diameter of <600 Å. Despite these measurements, the toroids released from T2 and T7 are considerably larger than the greatest dimensions of their respective heads. This paradox has been noted previously (Tikchonenko, 1975), but as mentioned above, has been attributed to an artifact of decondensation.

The discrepancy between phage head and toroid dimensions and the apparent flexibility of DNA toroids (Klimenko et al., 1967; Earnshaw et al., 1978) has suggested to us that a rearrangement of DNA after its expulsion from a phage head capsid does in fact occur, but this represents little more than the unfolding of an already existent toroid. (The term "folded toroid" will refer to a structure that has the potential to unfold and assume the shape of a toroid. This is distinct from the use of the same term by Serwer (1989) to describe a toroid that is generated by the cyclization of a rod-shaped structure.) To understand how a toroid might be folded within a phage head we draw upon our previous investigations of toroid formation in vitro.

Recently we presented support for a new model of DNA organization within toroidal condensates (Hud et al., 1995). In this model the DNA of a particular toroid is contained within a series of equally sized nonconcentric contiguous loops. An associated model for the kinetics of toroid formation indicates that each toroid begins as a single loop upon which successive loops are condensed in such a fashion that their centers are at gradually increasing radii from the toroidal axis. A fundamental implication of this model is that the diameter of the loops from which any toroid is constructed, the "toroid-loop" diameter, is equal to the sum of the toroid outer radius and the radius of the toroid hole. On the basis of this, the toroids of T2 and T7 seem to be initiated by DNA loops that are ~900 and 550 Å in diameter, respectively.

Our models for the structure and formation of DNA toroids, the calculated diameters of the putative T2 and T7 DNA toroid-loops, and the size of their respective heads suggest a possible origin of the folded toroid packaging motif. In this model, the first DNA to enter the phage head forms the initial loop of the toroid (Fig. 1 *A*). Formation of this initial loop has been implicated as a crucial factor in determining the ultimate dimensions of a toroid (Hud et al., 1995). Additional DNA then enters the head and associates with the initial and subsequent loops of the developing toroid. (For a review of proposed mechanisms for DNA translocation into the phage head and the generation of an environment within the phage head that is conducive to DNA condensation see Serwer, 1989.) As condensation proceeds, the toroid is expected to gyrate within the phage head so that the point of DNA addition onto the toroid is always near the proximal vertex of the head. Gyration of the toroid allows condensation to occur without entanglement of DNA within the phage head and without the generation of axial strain in the packaged DNA, two previously proposed obstacles to toroidal models (Serwer, 1986). As head filling proceeds, the outside diameter of the toroid will eventually become equal to the inside diameter of the maximally expanded phage head (Fig. 1 *B*). Continued condensation of DNA onto the toroid is then expected to cause the toroid to assume a bifolded close-packed state (Fig. 1 *C*).

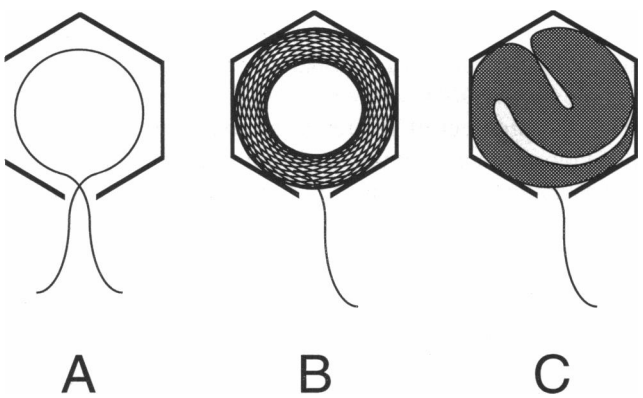

FIGURE 1 A model for the formation of folded DNA toroids within bacteriophage heads. (*A*) The first DNA to enter the phage head forms the initial loop of the toroid. (*B*) In the presence of a condensing environment, successive loops of DNA are deposited onto the initial loop, and the toroid develops as described by the constant radius of curvature model (Hud et al., 1995). The condensation of DNA, as it enters into the phage head, results in the gyration of the toroid such that the point of DNA deposition onto the toroid is always near the proximal vertex. (*C*) When the toroid has grown to a point at which it becomes constrained by the maximally expanded protein capsid, it begins to collapse upon itself. Head filling will continue until either the folded toroid is no longer able to gyrate within the protein capsid or nuclease cleavage of the entering DNA is triggered.

Head filling will continue until the folded toroid is too large to rotate and take on additional DNA or until endonuclease cleavage of the entering DNA is triggered or both (Casjens et al., 1992).

Here the dimensions of the bacteriophage T2 head and toroid are used to illustrate what we believe to be the result of the proposed in situ toroid formation process. A bifolded T2 toroid with circular cross sections has an approximate maximum length of 850 Å and an average width of 829 Å. Although these dimensions are quite close to the dimensions of a T2 head, this structure as strictly described would not be able to fit inside the icosahedral T2 protein capsid. However, the folded toroid is expected to fit if it is allowed to distort so as to conform to the shape of the phage head. In Fig. 2 we show a scale model T2 toroid that has been folded and distorted such that it will fit within the T2 protein capsid and not leave any appreciable voids near the top or bottom of the head. This is our model for the organization of DNA within T2.

Earnshaw and Casjens (1980) have presented several arguments in support of the hypothesis that all double-stranded DNA bacteriophages organize their genomes in a similar fashion. Considering their review and more recently reported experimental studies, we are inclined to agree. In light of this, our model for DNA organization within T2 is presented as a model representative to some degree of all double-stranded DNA bacteriophages.

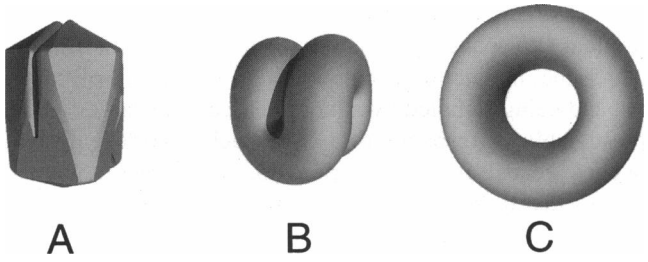

FIGURE 2 Space-filling models of toroids related to the packing of DNA by bacteriophage T2. A scale model of an expelled and unfolded T2 toroid is shown (*C*). This fully extended T2 toroid is too large to fit within a T2 head. If this toroid is bifolded it becomes considerably more compact (*B*). However, even when folded it has a slightly larger maximum diameter than a T2 phage head does. Furthermore, if a T2 toroid could be folded as such and fit within a T2 head, voids would be expected to be present at both the top and bottom of the head, features that have never been observed. The most consistent model for DNA organization seems to be a bifolded T2 toroid, which is distorted to the extent that it conforms to the shape of the T2 protein capsid (*A*). The crease shown in this model (*A*) is meant to illustrate that this structure developed from a folded toroid; however, the latter depositions of DNA onto the folded toroid are likely to fill in all available space so that such features may not actually be visible. The structures have been presented in this particular order to avoid the possible misconception that the process of head filling is accomplished by the folding of a fully formed toroid. The sequence of the models (from *A* to *C*) is most instructive when thought of as the unfolding of a toroid after it is released from a lysed phage head.

CONSISTENCY OF THE FOLDED TOROID MODEL WITH EXPERIMENTAL DATA

Thus far in the development of our model for DNA organization within bacteriophage heads we have sought only to be consistent with our studies of DNA toroids generated in vitro, the size of T2 and T7 toroids observed in lysates, and the dimensions of their respective heads. Furthermore, the scenario presented above for the formation of a folded toroid within the confines of a phage head will require experimental verification and refinement. This notwithstanding, the results from several structural studies outlined below demonstrate that the folded toroid model is in fact already consistent with a wide range of biophysical data.

Direct imaging of intact bacteriophage DNA

Electron microscopists have presented perhaps the most direct evidence of ordered DNA packaging within bacteriophage heads. Richards et al. (1973) reported circular striations with 25 Å periodicity in the heads of several different phages (P2, λ, T4, T5, T7) that had been disrupted just enough to allow the entrance of phosphotungstate. These observations led to the proposal of the ball-of-string model and further support of possible spool-like packing (Klimenko et al., 1967). A more recent analysis of intact phage-head DNA has been provided by the cryoelectron microscopy studies of Lepault et al. (1987). Their images of λ, T4, and T4 giant phages suggest considerable DNA organization in both circular and linear arrays. These regions of order, however, were never visible over an entire phage head, but were restricted to domains of local order that typically extend to ~200 Å (depending on phage). Furthermore, when multiple regions of order were observed within a single phage head, they were found to be in several distinct orientations. Lepault et al. (1987) also reported that DNA within prolate heads seemed to be preferentially oriented parallel to their long axis; however, the striations near the ends of even the greatly elongated T4 giant phages were frequently observed to follow a curved path defined by the ends of the capsid. On the basis of their study, Lepault et al. (1987) concluded that DNA organization within phage heads is neither random nor characterized by a clearly defined axis of symmetry.

The model for DNA organization shown in Fig. 2 *A* contains several regions of local DNA order that are spatially limited by the thickness of the toroid and directionally defined by toroid folding. Although these features are perfectly consistent with the general observations described in the preceding paragraph, our model for DNA organization is given even further support by close examination of the cryoelectron micrograph presented by Lepault et al. (1987) of the bacteriophage T4. In this micrograph (Fig. 3 *A*) the 25 Å striations near one end of the head follow a circular path defined by the capsid; however, linear striations that are parallel to the long axis of the phage seem to progress from near the middle of the phage head all the way to the opposite

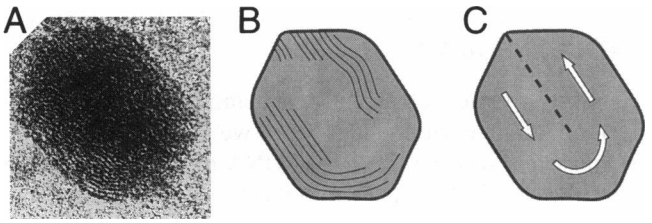

FIGURE 3 (*A*) Electron micrograph of a bacteriophage T4 embedded in vitreous ice (from Lepault et al., 1987, by permission of Oxford University Press). (*B*) A graphic representation of the most visible DNA paths in Fig. 3 *A*. (*C*) A folded toroid model of DNA organization within bacteriophage T4. The dashed line represents a boundary between regions of the folded toroid (see Fig. 2 *B*), a feature that may not actually be visible because of the high density of packing. The arrows represent the average paths of DNA molecules within the folded toroid. Note that these average paths are consistent with those observed in the electron micrograph.

end of the head. Although no previously proposed model predicts such a pattern, this is exactly what would be expected if our model were to be imaged in a particular orientation (Fig. 3 *C*).

Electron microscopy of lysed bacteriophages

Icosahedral-like (Ghosh et al., 1984), spheroidal (Richards et al. 1973), and toroidal (Klimenko et al. 1967; Richards et al., 1973) DNA structures have all been observed in the lysates of bacteriophage T2. Although the folded and distorted toroid model (Fig. 2A) may at first seem obscure, all three structures in Fig. 2 are perfectly consistent with these electron microscopy investigations. This suggests that the variety of observed morphologies for expelled DNA particles may actually be the progressive stages of toroid unfolding.

X-ray diffraction studies

X-ray diffraction studies have revealed a mean Bragg spacing of ~24 Å for DNA packaged within the heads of various wild-type bacteriophages (North and Rich, 1961; Earnshaw et al., 1976; Earnshaw and Harrison, 1977; Earnshaw et al., 1978). Although hexagonal packing has not been proven, it is assumed that this spacing corresponds to a interhelix distance of ~28 Å. This spacing is implicit in the model developed here, for the dimensions of the apparently intact T2 toroid were verified by comparing its volume with the volume of a T2 genome packed at a density corresponding to the interhelix distance of 28 Å.

Under some circumstances, the size of crystals within a sample can be determined by applying the fact that single crystal diffraction spots and crystalline powder diffraction rings become broader with decreasing crystal size (Guinier, 1963). On the basis of this principle, the width of the 24 Å diffraction band described above has led to the assignment of "apparent" domains of DNA order within several bacteriophage heads. These domains measure ~125 Å for T7 and λ (North and Rich, 1961; Earnshaw and Harrison, 1977), 145 Å for T2 and T4 (North and Rich, 1961; Earnshaw et al., 1978), and 214 Å for T4 giant phage (Earnshaw et al., 1978). The determination of an apparent domain size does not necessarily reveal the actual dimensions of crystallinity, unless the crystals are relatively free of defects and their morphology is well defined. Some investigators have favored the interpretation of line widths as representing the size of well defined, highly ordered domains of DNA packing (North and Rich, 1961; Earnshaw and Harrison, 1977), whereas others have interpreted line width as being simply an indication of the degree of order throughout the head (Earnshaw et al., 1978; Stroud et al., 1981).

The model presented here is consistent with the width of the 24 Å diffraction band being both a result of discrete crystalline domains and the degree of disorder within these domains. The thickness or cross-sectional diameter of a toroid in our model is a potential domain of DNA crystallinity. However, the diameters of these regions, 250 Å for T7 and 380 Å for T2, are considerably larger than the reported apparent domains. We believe that the reduction in the apparent domain size, i.e., diffraction line broadening, is a result of imperfect local DNA packing within these domains. This is supported further by the lack of higher orders of the 24 Å band in electron diffractograms of T4 giant phage (Lepault et al., 1987).

The 24 Å diffraction ring from a single crystal of T7 (North and Rich, 1961) and pellets of λ, P22, and T7 (Earnshaw and Harrison, 1977) have been shown to be modulated with spatial frequencies corresponding to the dimensions of their respective phage heads. Earnshaw and Harrison (1977) have attributed this modulation to the presence of ordered "shells" of DNA. However, we prefer the original explanation provided by North and Rich (1961) in which the modulation is considered to be a result of the 24 Å band being sampled by a larger lattice. This larger lattice presumably corresponds to a close-packed arrangement of phages heads. If this interpretation is correct, then the phages that do not exhibit a modulated 24 Å band may not do so simply because of a lack of regular packing order in high density gels.

Preferential alignment of DNA

Bendet et al. (1960) were the first to present evidence supporting the existence of regular DNA order within bacteriophage heads. They reported that samples of T2 exhibited negative birefringence when subjected to hydrodynamic forces, which presumably caused alignment of these prolate phage particles. This observation led them to conclude that DNA is at least partially oriented parallel to the long axis of T2. Gellert and Davies (1964) later approached this problem quantitatively with flow birefringence studies of T4, a phage that is morphologically similar and closely related to T2. Their results indicated that ~9% of T4 DNA is preferentially oriented parallel to the long axis. Addi-

tional optical dichroism investigations have confirmed the direction of preferentially aligned DNA within the T-even phages, with quantitative measurements ranging from 7–30% (Maestre, 1968; Basu, 1977). The optical properties of the smaller isometric phages T7, P22, ϕCd-1, and λ have been investigated similarly (Kosturko et al., 1979; Hall and Schellman, 1982a). These phages, however, are reported to exhibit positive birefringence, indicating a preferred orientation of DNA that is in this case perpendicular to the axis defined by the phage tail.

These optical absorption studies are limited in that they can only yield unambiguous results for the extreme cases in which DNA is organized either uniformly parallel or perpendicular to the long axis of a phage (Hall and Schellman, 1982b). Thus far no phage has been found to possess such a uniform alignment of DNA, making it impossible to assign a structure solely on the basis of optical absorption data. Accordingly, investigators who have used optical techniques have for the most part attempted to rule out or refine existing models (Kosturko et al., 1979; Hall and Schellman, 1982a,b). Similarly, we are able to state that the model structure presented here for T2 is at least qualitatively consistent with the optical data for the prolate phages, inasmuch as it predicts a negative birefringence resulting from a preferential alignment of DNA parallel to the long axis of the phage. Likewise, the positive birefringence of the smaller isometric phages could be a result of smaller toroids folding and orienting normal to the direction proposed here for T2.

Placement of genome loci with respect to the protein capsid

Several attempts have been made at revealing a possible relationship between phage genome loci and proximity to the protein capsid. The initial cross-linking study of λ by Widom and Baldwin (1983) indicated that no region of the phage genome is exempt from contact with the protein capsid. However, ion etching studies of T4 and λ (Black et al., 1985; Brown and Newcomb, 1986) demonstrated that the DNA which enters the phage head last is lost first when phage DNA particles are etched away from the outside to the inside. In reconciliation of these earlier investigations, a more recent and quantitative cross-linking study involving T7 (Serwer et al., 1992) demonstrated that although all regions of the genome are susceptible to capsid cross-linking, the probability of cross-linking the last 26% of the genome to enter the capsid is almost three times greater than the probability of cross-linking the first 25% to enter.

Illustrated in Fig. 4 are cut-away views of a hypothetical bacteriophage head during the head-filling process as proposed by our folded toroid model. In the early stages of head filling, the thin toroid is believed to be fully extended within the head (Fig. 4 *A*). As the toroid folds, DNA is continuously added to the outer surface of the folded toroid such that the DNA packaged during the early stages becomes preferentially sequestered toward the center of the head, whereas that packaged during the latter stages is closer to the protein capsid (Fig. 4, *B* and *C*).

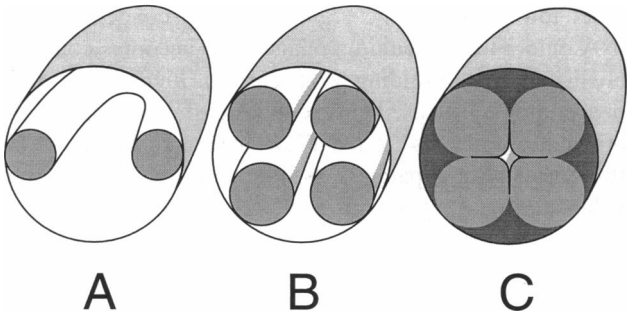

FIGURE 4 Illustrated cross sections of phage heads during the process of head filling. (*A*) In the early stages of DNA condensation, the developing toroid is expected to be fully extended. (*B*) As the toroid grows, the constraint imposed by the protein capsid causes it to fold. (*C*) Continued addition of DNA onto the folded toroid results in a compact structure that conforms to the shape of the protein capsid. The light gray region represents the protein capsid, and the medium and dark gray regions represent DNA that has entered during the early and late stages of head filling, respectively. This representation of the model serves to illustrate that the DNA that enters the head during the early stages of head filling (*A*) will be preferentially shielded from contacts with the protein capsid when head filling is complete (*C*).

Relative placement of genome loci

Before the protein-DNA cross-linking investigations, Cantor and co-workers began probing the packaged λ genome with DNA-DNA cross-linking agents in search of a possible regular spatial arrangement of genome loci (Haas et al., 1982; Welsh and Cantor, 1987). Their results indicated that regions corresponding to λ *Bgl*II and *Eco*RI restriction fragments can be cross-linked into all possible pairings regardless of their relative positions in the genome. Quantitative analysis of the frequency of restriction fragment-pair generation led to the conclusion that the actual organization of DNA is either complex, random, or variable among phages of the same type.

In our model for toroid structure, a particular loop of DNA within a toriod is not only in contact with the immediately preceding and following loops, but theoretically comes into close contact with a considerable number of loops that lie on both sides of its own plane (Hud et al., 1995). For example, in an idealized toroid with a loop size of 500 bp, a single loop has the potential to be in contact with (or cross-linked to) as many as 70 other loops of the toroid. This number of loops in such a toroid would span a region of DNA as great as 35 kb. Furthermore, three-dimensional toroid models reveal that the emergence of each loop at the outer edge of a toroid, combined with toroid folding, presents additional opportunities for the cross-linking of very distal regions of DNA. Although this assessment of potential cross-linking has been determined primarily on the basis of the DNA organization within a relaxed idealized

model toroid, it may be argued that the condensation of DNA onto a toroid within a phage head becomes considerably different after folding has occurred. However, this does not seem to be the case because DNA particles ejected from both heads of phages T2 and T7, as described above, unfold into toroids with apparently as little as 5% decondensation of their respective genomes.

Raman spectroscopy

Raman spectroscopy allows quantitation of the relative presence of well defined secondary structures in biological molecules and macromolecular assemblies. The sensitivity of this technique is such that alterations in the secondary structures of proteins and nucleic acids as small as 2% can be detected. Thomas and co-workers (1987) have used Raman spectroscopy extensively for the study of DNA packaging by bacteriophages. The most recent studies, and reportedly the most accurate to date, demonstrate that at least 98% of the packaged P22 genome exists in the B-form structure with respect to sugar-phosphate backbone torsional angles, basepairing, and only small perturbations in base-stacking (Aubrey et al., 1992; Reilly and Thomas, 1994). Furthermore, there is absolutely no evidence of protein capsid interactions with packaged DNA. In fact, the only significant alteration in the packaged P22 DNA spectra, with respect to P22 DNA in solution, seems to be a result of increased phosphate-cation interactions.

Time-resolved Raman spectroscopy has also been used by Reilly and Thomas (1994) to monitor the hydrogen isotope exchange dynamics of packaged phage DNA. This investigation revealed that P22 DNA in the packaged state, with respect to free P22 DNA in solution, exhibits a small retardation of the purine 8CH exchange rate and an acceleration of the imino (NH) and amino (NH_2) proton exchange rates for all bases. The retardation of 8CH exchange seems to be the result of steric shielding of the major groove from free solvent access, which is perhaps expected considering the high density of DNA packaging inside a P22 head (Earnshaw and Harrison, 1977). The accelerated exchange rates of the imino and amino protons is unexpected, however, in light of the above-described evidence for the highly conserved B-form secondary structure of packaged P22 DNA. Reilly and Thomas (1994) propose that this increase in isotope exchange rates represents the stabilization of the base pair open state of B-form DNA in packaged P22 DNA, a state that is presumed to be a transient state of free DNA in solution and is required for the exchange of protons involved in Watson-Crick hydrogen bonding (Englander and Kallenbach, 1984).

The implication of minimally disrupted B-form secondary structure is consistent with the data on DNA toroids formed in vitro (Gosule and Schellman, 1978) and suggests that the folded toroid is not distorted to such an extent that there is appreciable disruption of B-form DNA helices. The lack of interaction between the protein capsid and the packaged DNA lends support to the hypothesis that the growing DNA particle has the ability to rotate freely within the protein capsid during head filling. The alterations in the phosphate vibrations caused by increased counterion interaction is expected and should be considered somewhat independent of packaging models, for Wilson and Bloomfield (1979) demonstrated that increased DNA phosphate charge neutralization is intimately associated with the condensation phenomenon. With respect to the accelerated isotope exchange rates of packaged T7 DNA, Reilly and Thomas (1994) reported that the proposed base-pair openings are apparently distributed throughout the phage genome rather than localized, such as at discrete kinks. They further suggest that the origin of this phenomenon is the result of divalent cation binding and the smooth bending of packaged T7 DNA. The model presented here is consistent with either explanation.

Chemical probing of phage DNA secondary structure

Chemical reagents that modify DNA bases have been used to probe the secondary structure of packaged phage DNA (Tikchonenko, 1975; Serwer, 1989). The reagents used in these studies (e.g., bisulfite, glyoxal, and methylhydroxylamine) typically react with the bases of single-stranded DNA but have reduced or undetectable reactivities with the bases of B-form DNA. The most comprehensive of these investigations has shown that ~20% of the DNA packaged within S_d (a coliphage similar in morphology to T7) can be modified by reagents that attack only regions of DNA with disrupted base-stacking (Tikchonenko, 1975). However, the same phage when exposed to nitrous acid exhibits an initial lag before the onset of base deamination, with respect to free single- and double-stranded DNA in solution (Tikchonenko, 1975). This, on the other hand, indicates that packaged S_d DNA contains no free amino groups before nitrous acid treatment. In an attempt to reconcile these data, it has been suggested that 20% of packaged S_b DNA exists in a state of disrupted helices, with base amino groups being protected from deamination by the formation of hydrogen bonds with capsid proteins (Tikchonenko, 1975; Serwer, 1989).

Equilibrium binding studies of the intercalator ethidium have also been used to probe the secondary structure of phage DNA. Analysis of Scatchard plots suggests that although the majority of packaged T7 DNA has a lower affinity for ethidium than does free DNA in solution, ~1.2% of the T7 genome contains what are considered to be high-affinity binding sites (Griess et al., 1985). These sites exhibit a 50% increase in the magnitude of the negative ΔH of binding over that of free DNA. Similar findings were reported for packaged P22 DNA (Griess et al., 1986). Although the origin of these high-affinity sites has not been proven, Griess et al. (1985) proposed that they are the result of sharp turns, or kinks, in DNA helices.

The inference that a significant percentage of phage DNA exists in the form of disrupted helices with amino groups hydrogen-bonded to capsid proteins is completely inconsistent with the Raman spectroscopy studies described above. A possible reconciliation of this apparent contradiction between the Raman spectroscopy and chemical modification results may reside in the proposal that the smooth bending of packaged phage DNA causes small but detectable alterations in helix structure (Reilly and Thomas, 1994). If this is truly the case, then DNA bending may cause perturbations in helix structure that are large enough to allow chemical modification by reagents sensitive to base destacking but are too small to expose amino groups to nitrous acid attack. Such an explanation would also be consistent with the model presented here. The origin of the high-affinity ethidium binding sites, however, remains obscure with respect to the present model.

A COMPARISON WITH PREVIOUS MODELS

Most models for the organization of double-stranded DNA within bacteriophage heads have been developed on the basis of only a subset of the presently available physical data. Although it may not be possible to develop a single model that is simultaneously consistent with all data from every type of phage, we believe that the model presented here unifies a range of data that is at least as extensive as that accomplished by any previous model. As a corollary to this success, the folded toroid model possess features that are common to other models. Most obvious is the toroid morphology that is shared with the spool model; however, several other features are dissimilar to those of the spool model but are similar to those of other models. Here we briefly illustrate this point with a few examples.

Within the confines of a phage head, a folded DNA toroid will seem to be a dense particle with circular and linear striations near the surface. This is consistent with the original (and perhaps only) support for the ball-of-string model, which was the direct observation of circular striations within the minimally disrupted phage heads (Richards et al., 1973).

Serwer (1986) has proposed that DNA helices within phage heads change direction frequently at abrupt turns, or kinks, so that the DNA is essentially folded into parallel arrays as it enters the phage head. Refinements to the original model (Serwer, 1989) define the resulting structure of packaged DNA to be one or more toroid-like structures. These structures, however, differ significantly from the toroids discussed in the present work. The toroids used by Serwer in the modeling of DNA packaging are structured on the basis of an earlier proposal that toroids are actually rod-shaped structures that can become cyclized (Eickbush and Moudrianakis, 1978). This notwithstanding, the resulting DNA paths for a particular interpretation of the Serwer model could closely resemble those in the model presented here. If the toroids within phage heads are as Serwer suggests, then perhaps additional experiments similar to those of Kilmenko et al. (1967) and Richards et al. (1973) could be performed under conditions that favor increasing levels of DNA decondensation. If decondensing phage toroids are found to assume rod-shaped structures, then support would be given to the model by Serwer rather than to the present model.

On the basis of their cryoelectron microscopy studies, Lepault et al. (1987) proposed the nematic liquid crystal model for DNA organization within bacteriophage heads, which is akin to the liquid crystals formed by high concentrations of DNA in vitro (Rill, 1986). Although this model is in agreement with their micrographs, it does not specifically explain the relationship between localized regions of order observed within single phage heads or the origin of several other properties of packaged phage DNA described above. The folded toroid model presented here, however, is also consistent with the images presented by Lepault et al. (1987). Accordingly, the close packing and local order of DNA in the folded toroid model may actually be viewed as a special case of the liquid crystal model, a case in which the relative placement of ordered regions is governed by the topology of the folded toroid.

In the rather particular case of the greatly elongated bacteriophage T4 giant, Earnshaw et al. (1978) suggested that DNA is organized within an oval-shaped toroid. Considering their electron micrographs, those of Lepault et al. (1987), the dimension of a T4 giant head, and the size of the toroids they contain (Earnshaw et al., 1978), we are inclined to agree. At first, an oval toroid for DNA organization within T4 giant might be viewed as significantly different from the model developed above for T2; however, both models are consistent with our most fundamental hypothesis. Explicitly, toroids are the universal packing mechanism for double-stranded DNA within bacteriophage heads, but the actual shape or folding of a toroid within a particular phage head is dictated by both the dimensions of the DNA toroid and the protein capsid within which it is confined.

I thank Drs. Rod Balhorn and Ken Downing for many helpful discussions, the reviewers of this manuscript for valuable suggestions, and Joe D. Lee for assistance in the generation of computer graphics. This work was performed at the Lawrence Livermore National Laboratory under the auspices of the U. S. Department of Energy and was supported by Contract W-7405-ENG-48 and Laboratory Directed Research and Development Award 93-DI-003.

REFERENCES

Arscott, P. G., A.-Z. Li, and V. A. Bloomfield. 1990. Condensation of DNA by trivalent cations. 1. Effects of DNA length and topology on the size and shape of condensed particles. *Biopolymers*. 30:619–630.

Aubrey, K. L., S. R. Casjens, and G. J. Thomas, Jr. 1992. Secondary structure and interactions of the packaged dsDNA genome of bacteriophage P22 investigated by Raman difference spectroscopy. *Biochemistry*. 31:11835–11842.

Basu, S. 1977. Molecular arrangement of DNA in bacteriophage T4. *Biopolymers*. 16:2299–2314.

Bendet, I. J., D. A. Goldstein, and M. A. Lauffer. 1960. Evidence for internal organization of nucleic acid in T2 bacteriophage. *Nature*. 187: 781–782.

Black, L. W., W. W. Newcomb, J. W. Boring, and J. C. Brown. 1985. Ion etching of bacteriophage T4: support for a spiral-fold model of packaged DNA. *Proc. Natl. Acad. Sci. USA*. 82:7960–7964.

Brown, J. C., and W. W. Newcomb. 1986. Ion etching of bacteriophage λ: evidence that the right end of the DNA is located at the outside of the phage DNA mass. *J. Virol.* 60:564–568.

Casjens, S., W. Wyckoff, M. Hayden, L. Sampson, K. Eppler, S. Randall, E. T. Moreno, and P. Serwer. 1992. Bacteriophage P22 portal protein is part of the gauge that regulates packing density of intravirion DNA. *J. Mol. Biol.* 224:1055–1074.

Earnshaw, W. C., and S. R. Casjens. 1980. DNA packaging by the double-stranded DNA bacteriophages. *Cell*. 21:319–331.

Earnshaw, W. C., and S. C. Harrison. 1977. DNA arrangement in isometric phage heads. *Nature*. 268:598–602.

Earnshaw, W., S. Casjens, and S. C. Harrison. 1976. Assembly of the head of bacteriophage P22: x-ray diffraction from heads, proheads and related structures. *J. Mol. Biol.* 104:387–410.

Earnshaw, W. C., J. King, S. C. Harrison, and F. A. Eiserling. 1978. The structural organization of DNA packaged within the heads of T4 wild-type, isometric and giant bacteriophages. *Cell*. 14:559–568.

Eikbush, T. H., and E. N. Moudrianakis. 1978. The compaction of DNA helices into either continuous supercoils or folded-fiber rods and toroids. *Cell*. 13:295–306.

Englander, S. W., and N. R. Kallenbach. 1984. Hydrogen exchange and structural dynamics of proteins and nucleic acids. *Q. Rev. Biophys.* 4:521–655.

Gellert, M., and D. R. Davies. 1964. Organization of DNA in bacteriophage T4. *J. Mol. Biol.* 8:341–347.

Ghosh, A. N., A. Sen, and N. N. Das Gupta. 1984. Packing pattern of DNA in bacteriophage T2. *Z. Naturforsch. Sect. C Biosci.* 39:692–694.

Gosule, L. C., and J. A. Schellman. 1976. Compact form of DNA induced by spermidine. *Nature*. 259:333–335.

Gosule, L. C., and J. A. Schellman. 1978. DNA condensation with polyamines. I. Spectroscopic Studies. *J. Mol. Biol.* 121:311–326.

Griess, G. A., P. Serwer, and P. M. Horowitz. 1985. Binding of ethidium to bacteriophage T7 and T7 deletion mutants. *Biopolymers*. 24: 1635–1646.

Griess, G. A., P. Serwer, and P. M. Horowitz. 1986. Binding of ethidium to bacteriophages T7 and P22. *Biophys. J.* 49:19–21.

Guinier, A. 1963. X-Ray Diffraction in Crystals, Imperfect Crystals and Amorphous Bodies. W. H. Freeman and Co., San Francisco.

Haas, R., R. F. Murphy, and C. R. Cantor. 1982. Testing models of the arrangement of DNA inside bacteriophage λ by crosslinking the packaged DNA. *J. Mol. Biol.* 159:71–92.

Hall, S. B., and J. A. Schellman. 1982a. Flow dichroism of capsid phages. II. Effect of DNA deletions and intercalating dyes. *Biopolymers*. 21: 2011–2031.

Hall, S. B., and J. A. Schellman. 1982b. Flow dichroism of capsid DNA phages. I. Fast and slow T4B. *Biopolymers*. 21:1991–2010.

Harrison, S. C. 1983. Packaging of DNA into bacteriophage heads: a model. *J. Mol. Biol.* 171:577–580.

Hud, N. V., K. H. Downing, and R. Balhorn. 1995. A constant radius of curvature model for the organization of DNA in toroidal condensates. *Proc. Natl. Acad. Sci. USA*. 92:3581–3585.

Klimenko, S. M., T. I. Tikchonenko, and V. M. Andreev. 1967. Packing of DNA in the head of bacteriophage T2. *J. Mol. Biol.* 23:523–533.

Kosturko, L. D., M. Hogan, and N. Dattagupta. 1979. Structure of DNA within three isometric bacteriophages. *Cell*. 16:515–522.

Lepault, J., J. Dubochet, W. Baschong, and E. Kellenberger. 1987. Organization of double-stranded DNA in bacteriophages: a study by cryo-electron microscopy of vitrified samples. *EMBO J.* 6:1507–1512.

Maestre, M. F. 1968. Transient electric birefringence studies of T2 bacteriophage and T2 ghost. *Biopolymers*. 6:415–430.

North, A. C. T., and A. Rich. 1961. X-ray diffraction studies of bacterial viruses. *Nature*. 191:1242–1245.

Reilly, K. E., and G. J. Thomas, Jr. 1994. Hydrogen exchange dynamics of the P22 viron determined by time-resolved Raman spectroscopy. *J. Mol. Biol.* 241:68–82.

Richards, K. E., R. C. Williams, and R. Calendar. 1973. Mode of DNA packing within bacteriophage heads. *J. Mol. Biol.* 78:255–259.

Rill, R. L. 1986. Liquid crystalline phases in concentrated aqueous solutions of Na+ DNA. *Proc. Natl. Acad. Sci. USA*. 83:342–346.

Serwer, P. 1986. Arrangement of double-stranded DNA packaged in bacteriophage capsids: an alternate model. *J. Mol. Biol.* 190:509–512.

Serwer, P. 1989. Double-stranded DNA packaged in bacteriophages: conformation, energetics, and packaging pathway. *In* Chromosomes: Eukaryotic, Prokaryotic, and Viral. K. N. Adolph, editor. CRC Press, Boca Raton. 203–223.

Serwer, P., S. J. Hayes, and R. H. Watson. 1992. Conformation of DNA packaged in bacteriophage T7. Analysis by use of ultraviolet light-induced DNA-capsid cross-linking. *J. Mol. Biol.* 223:999–1011.

Stroud, R. M., P. Serwer, and M. J. Ross. 1981. Assembly of bacteriophage T7. Dimensions of the bacteriophage and its capsids. *Biophys. J.* 36: 743–757.

Thomas, Jr., G. J.. 1987. Biological applications of Raman spectroscopy, Vol. 1. *In* Raman Spectra and the Conformations of Biological Macromolecules. T. G. Spiro, editor. Wiley-Interscience, New York. 135–201.

Tikchonenko, T. I. 1975. Structure of viral nucleic acids in situ. *In* Comprehensive Virology 5. H. Fraenkel-Conrat and R. R. Wagner, editors. Plenum Press, New York. 1–117.

Welsh, J., and C. R. Cantor. 1987. Studies on the arrangement of DNA inside viruses using a breakable bis-psoralen crosslinker. *J. Mol. Biol.* 198:63–71.

Widom, J., and R. L. Baldwin. 1983. Tests of spool models for DNA packaging in phage Lambda. *J. Mol. Biol.* 171:419–437.

Wilson, R. W., and V. A. Bloomfield. 1979. Counterion-induced condensation of deoxyribonucleic acid: a light-scattering study. *Biochemistry*. 18:2192–2196.