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Packaging of DNA into Bacteriophage Heads:

A Model

A model is suggested for the geometry of DNA entry into a bacteriophage head. It accounts for recent observations indicating absence of a unique, ordered sequence of windings in the packaged DNA.

The heads of bacteriophages such as λ, P22 and T4 contain a very compactly packaged single molecule of double-stranded DNA. Electron microscopy of gently disrupted particles (Richards *et al.*, 1973; Earnshaw *et al.*, 1978) and X-ray scattering from isometric phages in solution (Earnshaw & Harrison, 1977) both indicate that the DNA is wound into a spool-like structure. Spatially adjacent segments of the molecule are forced by tight packing to lie parallel to each other, and the relatively regular intersegment spacing is simply governed by the total length of the DNA molecule and the total volume available to it. For example, deletion mutants of bacteriophage λ that contain less DNA than wild-type phage have a correspondingly larger spacing between windings (Earnshaw & Harrison, 1977).

A series of recent experiments with bacteriophage λ, in particular those reported in this issue by Widom & Baldwin (1983), suggest that the detailed path of winding of the DNA molecule is not exactly determined, but rather that it can vary from particle to particle (see also Haas *et al.*, 1982). These experiments, which depend on the unique (rather than circularly permuted) sequence of the packaged λ genome, show that the distribution of sequences lying adjacent to the protein shell is uniform along the molecule, and that the neighborhood of any given sequence does not appear to be unique. Other relevant observations are that one end of the DNA, the last to enter the head, is attached to the tail in phages P2, P4 and λ (Chattoraj & Inman, 1974), and that in tailless capsids of P2, the cohesive ends can join to form a molecule with complex topological knots (Liu *et al.*, 1981).

These geometrical properties of the packaged DNA must be consequences of the packaging mechanism, an ATP-dependent insertion of DNA into a preformed prohead (Earnshaw & Casjens, 1980). The variation of spacing between windings with total DNA packages shows that ATP is needed at least in part to overcome repulsion of adjacent segments, and it strongly suggests that the spool must tighten up during the course of packaging. Such tightening is inconsistent with a fixed order for laying down successive windings of the spool. Packaging must nonetheless proceed in a manner that guarantees no entanglement during unpackaging. This note suggests a picture for the topology of packaging that satisfies these requirements.

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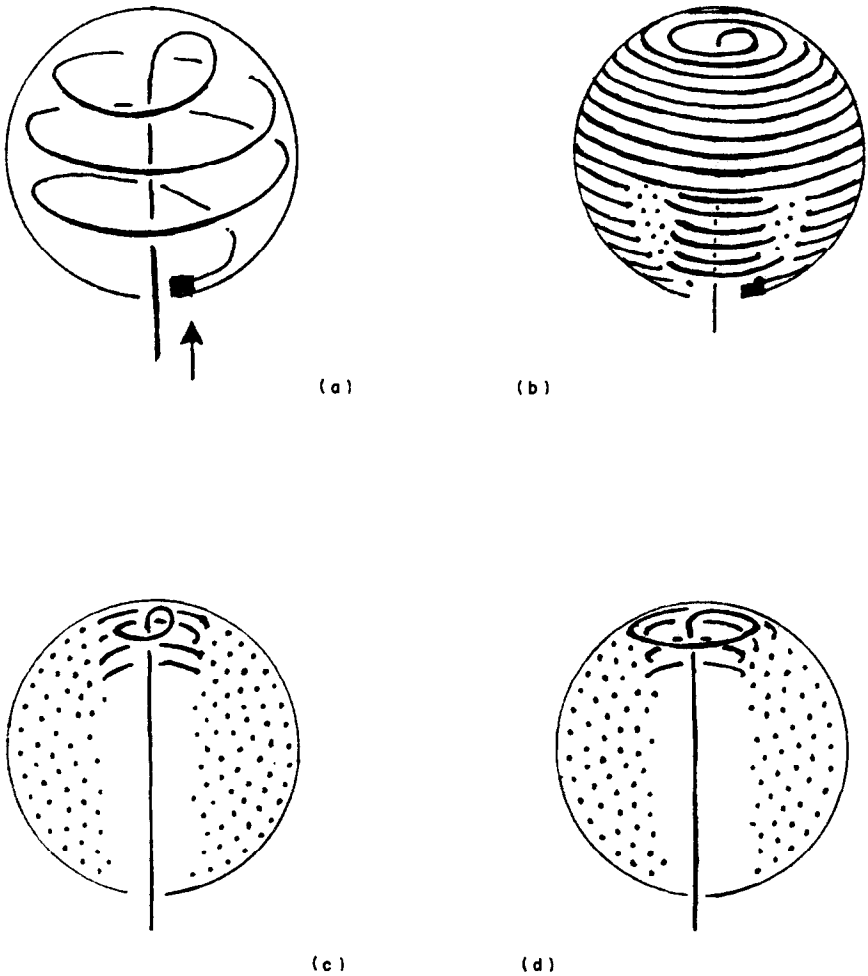

FIG. 1. (a) Proposed geometry for entry of DNA into a bacteriophage head. The leading end is attached near the point of entry. The hand of the supercoil is not known. (b) A packaged spool of intermediate tightness. (c) and (d) Addition need not proceed in any fixed order. Further addition to the spool in (b) can occur by adding a winding to the center or to the outside.

The basic features of the model are shown in Figure 1(a). The leading end of the DNA molecule is attached to the head near the point of entry. DNA enters axially and adds windings to the far end of the growing spool. In order to relieve twist, the head could rotate with respect to the entering strands. Alternatively, the DNA might attach to an "adaptor" anchored at the entry point but free to rotate with respect to the head. At an intermediate stage in packaging, there will be a moderately wound coil with roughly equally spaced turns (Fig. 1(b)). Further packaging forces this coil to tighten, and the tightening can occur in a number of ways depending on fluctuations from head to head. Layers can add near the axis (Fig. 1(c)) or wrap around the outside of earlier ones (Fig. 1(d)). Whatever

the detailed course of the coiling, a fundamental property is that the existing spool must be able to tighten. Adjacent windings must slide over each other and over the inside surface of the head.

In this model, entanglement is prevented by tethering the leading end and by inserting DNA along the axis of the spool. The absence of these features can lead to snarls when DNA leaves the head. Unpackaging can occur at any point just by reversing the sequence of entry, precisely what would occur if ATP-driven packaging stopped and DNA were allowed to eject. The final spool need not be strictly orthogonal to the axis: individual groups of turns could tilt (although local parallelism tends to be enforced by tight packing at late stages), or the whole spool could twist somewhat, like a skein of wool. Electron micrographs indeed suggest that such twisting may occur in T4 giants, where most of the DNA lies roughly parallel to the long axis (see Earnshaw *et al.*, 1978).

By avoiding entanglement without requiring any particular ordered sequence of turns, the proposed model accounts for the observations of Widom & Baldwin (1983), that any sequence in λ can be adjacent to the wall of the head, and for the results of Haas *et al.* (1982), that any restriction fragment crosslinks equally well with any other. It accounts for the formation of complex knots in P2, by postulating spatial proximity of leading and trailing ends of the DNA (Liu *et al.*, 1981).

Any detailed view of a mechanism needs characterization of the DNA "pump". Hendrix (1978) has suggested a specific picture for a rotating connector, exploiting the symmetry mismatch of head and tail. A significant question is whether axial entry can be ensured by structures at the point of packaging, relying on the stiffness of DNA to create the geometry of Figure 1, or whether an axial guide is required. Whatever the specific mechanism, the proposed model satisfies the biological requirements of reversible winding without imposing more order than necessary. The absence of non-essential order appears to be an important conclusion from the recent experimental observations.

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