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MATURATION OF THE HEAD OF BACTERIOPHAGE T4

V. A Possible DNA Packaging Mechanism: In Vitro Cleavage of the Head Proteins and the Structure of the Core of the Polyhead

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The most recent developments in studies on the maturation of the head of bacteriophage T4 are described and discussed.

The major features of the maturation steps of the head are the following: a) The viral DNA is pulled into an empty head in a series of events. b) Cleavage of two core proteins, P22 (MW = 31,000), to small fragments and the internal protein IPIII (MW = 23,000) to IPIII* (MW = 21,000) appears to be intimately linked to the DNA packaging event, whereas the cleavage of the major head protein of the viral coat, P23 (MW = 55,000), to P23* (MW = 45,000) precedes the DNA packaging event.

The P22 core proteins appear to be the precursors of the well-known, highly acidic internal peptides. We have tested the idea that these internal peptides collapse DNA by a repulsive interaction as various polymers like polyethylene oxide (PeO) and polyacrylate (PAA) do. We found that high concentrations of the internal peptides, polyaspartic acid, and polyglutamic acid, collapse DNA. This supports the idea that repulsive interactions with the internal peptides may collapse the DNA inside the head, and thus pull the DNA in.

The structure of the DNA collapsed by PeO was studied with the electron microscope and contrasted with the structure of DNA collapsed by polylysine. We find PeO collapses T4 DNA into compact particles best described as a ball of string, of about the size of the T4 head. Two structures are seen in preparations of polylysine-collapsed DNA. One has the shape of a donut and the DNA strand appears to be radially distributed as a spiral; the other is a stemlike structure in which the DNA is folded back and forth in a pleated structure.

The aberrant tubular polyhead contains the precursor protein P23, P22, and the internal proteins IPIII and IPII. Addition of chloroform to a polyhead preparation extracts the proteins P22, IPIII, and IPII. This removes the inside material (core) seen in polyheads prior to the chloroform extraction, as judged by electron microscopy. We conclude that P22, IPIII, and IPII (and supposedly IPI) are the major structural constituents of the core of polyheads, while P23 is the major constituent of the outer coat.

Structural studies reveal that the core of the polyhead is highly organized into a helical structure consisting of 4–6 helical chains wound about a hollow center of approximately 150 Å diameter.

Cleavage of the various head proteins occurs when partially purified polyheads are incubated at 37°C. In a 100 minute incubation, about 60–70% of P23 (MW = 55,000) is converted to P23* (MW = 45,000) and a significant conversion of IPIII (MW = 23,000) to IPIII* (MW = 21,000) is seen. The protein P22 (MW = 31,000) disappears during this incubation and is supposedly cleaved to small fragments. The in vitro products, P23* and IPIII*, have the same molecular weight as the in vivo products, suggesting that the protease cleavage is specific. However, several other protein fragments are generated during the in vitro cleavage reaction which have not been observed in vivo. Appropriate mutant studies reveal that the products of genes 21 and 22 are required for these in vitro cleavage reactions.

INTRODUCTION

Genes Involved in the Early Assembly Steps of the Head (Group A)

Of the 46 known genes which control phage assembly, 17 are known to be involved in the formation of the head (1, 2). These genes can be ordered into two groups. Group A, containing genes 20, 21, 22, 23, 24, 31, and 40 (1–3), is involved in the early steps of head formation. The genes in group B (genes 2, 4, 13, 14, 16, 17, 49, 50, 64, and 65) control later steps of head formation (2). The genes in group A will be discussed first. Many code for structural proteins of the head and determine the size and shape of the head, since a mutation in any one of them (with the exception of gene 23) results in the accumulation of head-related assembly products. We have tried to assemble in Fig. 1 the relevant information regarding the genes in group A. The diagram illustrates the head-related structures produced when a particular gene is defective. An arrow to the left of a box points to the observable result of a defect in that gene. The diagram also demonstrates cooperation of the genes in the assembly of the various structures. The protein constituents of the various particles are also included.

Gene 23. The product of gene 23 (P23) is the major structural protein of the head (4–6); it has a molecular weight of about 55,000 daltons. No head-related particles accumulate in cells infected with a phage containing an amber mutation in gene 23. The altered P23 product synthesized by different temperature-sensitive mutants in gene 23, however, assembles at restrictive temperature into malformed heads of various shapes which appear to contain no DNA (3, 5).

Some mutations are known in gene 23 which affect the length but not the diameter of the phage head (7, 8). Several mutants produce shorter than normal heads (Fig. 1).

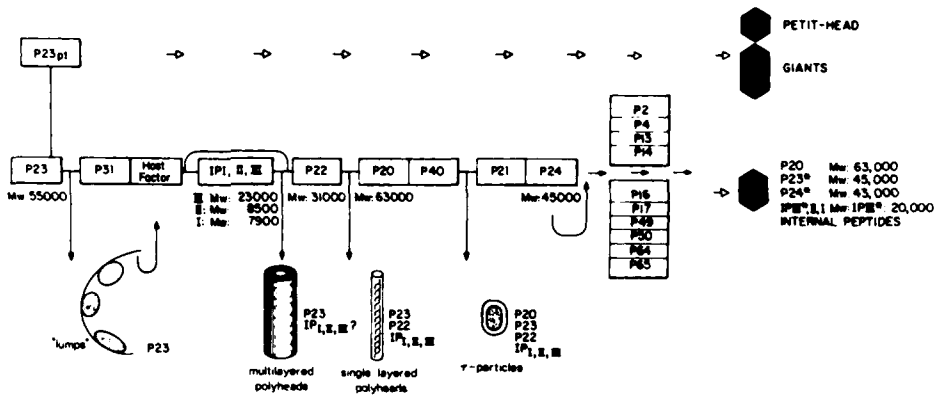


Fig. 1. Cooperation of the genes in group A in the formation of the various head-related structures. This diagram illustrates the head-related structures produced when particular genes are defective. This is an updated version of an earlier presentation (3) and includes work from different laboratories (see text). An arrow to the left of a box points to the observable result of a defect in that gene. Genes to the left of an arrow of a particular structure are essential for the formation of this structure and genes to the right are not. The internal protein genes (IPI, II, and III) are the exception to this organization of the diagram, since their function is absolutely required for multilayered polyhead formation only. This is indicated by the line bypassing these genes. The structural components of the various structures are also listed.

Most of these structures are aberrant assembly products with the exception of the "lumps" and the τ-particles found in 24 defective cells as indicated by the arrow pointing away from these structures.

The box containing $P23_{pt}$ illustrates that mutations (pt) are known in gene 23 which result in the formation of shorter than normal (petit head) and giant heads.

A significant fraction of the particles in these lysates have heads of normal length, but some mutants produce in addition exceptionally long heads (giants). Earlier mapping data located one of the mutants (pt E920g) in gene 66 between genes 23 and 24 (7). More recent data, however, locate this mutation in gene 23 (8). These data clearly show that P23 is one of the proteins involved in the system which controls the length of the capsid. Of course, one would expect that other proteins are implicated in this control.

The major head protein, P23 (MW = 55,000), is cleaved into a protein called P23* (MW = 45,000) during normal head maturation (17, 20, 36, 37). Cleavage of P23 and the other head proteins (P22, P24, and IPIII) discussed below is strongly inhibited by mutations in genes of group A (17, 20, 37). Thus, the various head-related structures produced in these mutant infected cells consist mainly of the precursor proteins. The fragment (expected MW = 10,000) cleaved off P23 is derived from the N-terminal end of P23 (17, 69) but is not found in the mature phage, which suggests that this cleavage fragment is degraded to very small peptides or amino acids.

Gene 31 and the host factor. In gene 31 defective cells, aggregates of the major protein P23 are formed. In thin sections of infected cells these apparently amorphous aggregates (called "lumps") can be observed adhering to the cell envelope (9).

Temperature shift experiments with ts-mutants of gene 31 have shown that the "lumps" dissolve, at least in part, if P31 is reactivated, and that this material can be used for head formation (10, 11). We therefore proposed that the product of gene 31 may be needed to protect P23 from random aggregation (solubility factor), or may directly con-

trol an early step of its polymerization (10). Gene 31 may have a role similar to genes 57 and 38. These genes are involved in tail fiber assembly, but are not found as structural components of the tail fibers. They appear to promote noncovalent dimerization of the tail fiber polypeptides (47).

A host factor defined by bacterial mutants (called groE or mop) is known which interferes with the action of gene 31 (11–13). Infection of such bacterial mutants with the wild-type phage leads to the expression of a similar phenotype as described for gene 31 defective cells. Moreover, these authors found T4 mutants which overcome the block of these bacterial strains and showed that these mutants map in gene 31. This demonstrates that gene 31 cooperates with a bacterial host factor(s) in some early step of head assembly (Fig. 1). It is noteworthy that gene 31 appears to act catalytically (10, 14), but the product of gene 31 has not been found.

The internal protein genes. The mature phage contains several proteins which are associated with the DNA. These so-called internal proteins were first discovered by Hershey (15) and were extensively characterized by Black and Ahmad-Zadeh (16). The internal proteins IPIII*, IPII, and IPI are basic and have molecular weights of 21,000, 8,500, and 7,900, respectively (16). The largest internal protein, IPIII*, is derived from a precursor protein IPIII (MW = 23,000) by cleavage (17). Black (18) has recently identified and mapped the structural genes for these internal proteins. They are located between genes e and 57. Most interestingly, he discovered that these proteins are not absolutely essential components of the head. Active phage production proceeds, although inefficiently, in cells infected with a phage carrying mutations in one or more of the internal protein genes. This fact is illustrated in Fig. 1 by a line which bypasses the internal proteins. Note, however, that IPIII, at least, is essential for the formation of the multilayered polyhead (18, 19).

Gene 22. Gene 22 codes for a protein with a molecular weight of about 31,000 daltons (17, 20). If gene 22 is defective, open-ended tubular structures accumulate, 60–70% of which consist of several concentric layers (3). These structures, called multilayered polyheads, are also observed in small amounts in gene 20 defective cells. They fall apart into individual tubes if isolated (22).

Studies with appropriate double mutants (3) determined that genes listed to the left of gene 22 in Fig. 1 are required for multilayered polyhead formation, while those listed to the right of gene 22 need not be functional. The essential genes are 23, 31, the host factor, and the internal proteins. The requirement of the internal proteins for multilayered polyhead formation was established by Showe and Black (19). More recently, Black (18) has shown that apparently only one of the internal proteins, IPIII, is required. The observation that the internal proteins are not essential for active production, but are required for multilayered polyhead formation in 22 defective cells, is extremely interesting. It has been suggested that the internal proteins may substitute to a certain degree for the role of gene 22 (19).

We have proposed that P22 is involved in the initiation and size determination process of the head (3) since the absence of gene 22 results in the formation of multilayered rather than single-layered polyheads (see below: Genes 20 and 40). This interpretation fits nicely with our more recent data (23, 24), which demonstrates that P22 and the internal proteins are the major structural components of the core of the polyheads (see below: The structural components of the polyhead core). This conclusion is also compatible with the suggestion that P22 and the internal peptides form a so-called assembly core (19). The core of the polyheads can be observed in thin sections of infected cells

(25) and has a highly ordered helical structure if isolated under appropriate conditions (26) (see below: The structure of the polyhead core).

Purified multilayered polyheads contain only the protein P23 (23) (Fig. 1 and Table I). We have not detected the internal proteins although the genetic data (18, 19) show that at least one of the internal proteins (IPIII) is required for multilayered polyhead formation. It is possible that IPIII is lost during the purification procedure or, alternatively, it may be a transient component.

The protein P22 (MW = 31,000) is cleaved during normal head maturation to small fragments (17, 20) and may be the precursor of the internal peptides (19, 27). We have shown that the cleavage of P22 appears to be linked to the DNA-packaging event (28) (see below: Pathway – the head maturation steps).

Genes 20 and 40. Gene 20 codes for a protein of about 63,000 daltons found in the head, but the product of gene 40 has not been found (17). In 20 or 40 defective cells, tubular single-layered polyheads accumulate (3, 5, 29). These polyheads have the diameter of the innermost layer of the multilayered polyheads observed in 22 defective cells (22). The shell of the polyhead is composed of subunits (mostly P23) arranged on a hexagonal net which has been studied in detail by electron microscopy and optical diffraction (30–33).

Genes listed to the left of genes 20 and 40 in Fig. 1 are required for single-layered polyhead assembly (3), with the exception of the internal protein genes (18, 19), while the genes to the right are not essential. Since the surface structure of polyheads and τ-particles are very much alike (34, 35), one may regard the τ-particles as polyheads of a defined length containing hemispherical caps. Genes 20 and 40 are required for τ-particles, but not for single-layered polyhead formation, and we proposed for this reason that genes 20 and 40 are involved in the formation of the hemispherical caps of the τ-particles (3).

Isolated polyheads contain the proteins P23, P22, and the internal proteins IPIII, IPII, and possibly IPI (23) (Fig. 1 and Table I). Chloroform extracts the core protein P22 and the internal proteins from the polyheads (23), which explains why earlier data indicated that P23 is the sole polyhead component (36).

Genes 21 and 24. The protein of gene 21 has not been found, but gene 24 codes for a protein of molecular weight about 45,000 daltons (17). P24 is also cleaved during normal head formation to P24* (43,000) (17). In 21 or 24 defective cells so-called τ-particles accumulate (1, 3, 25). These particles are somewhat smaller than the mature T4 head (25). Isolated particles contain no measurable amount of DNA (24, 38) and they have a surface structure similar to that of polyheads (34). Somehow gene 24 affects the head surface structure since the mutation conferring osmotic-shock resistance to the phage maps in gene 24 (39). Other data suggest that gene 21 codes for a protein which may be the proteolytic enzyme which cleaves protein P22 (19, 23) (see below: In vitro cleavage of the head proteins of partially purified polyheads).

The τ-particles isolated from 21 defective cells contain the precursor protein P23 and also P22, IPIII, IPII, IPI, and P20 (24, 38, 40). The particles isolated from 24 defective cells have a similar composition (Laemmli, unpublished).

Recent data show that the τ-particles formed at nonpermissive temperature in cells infected with a ts-mutant in gene 24 can be converted into active phage particles if permissive temperature is established (41). [Similar experiments with 21 infected cells were negative (24, 38, 40)]. An important control established that the τ-particles remain intact and do not disperse and reassemble following activation of gene 24 (43). We also found that a very large amount of DNA made after activation of P24 is incorporated into

TABLE I. Summary of Protein Constituents of Various Polyheads and Their in Vitro Cleavage Capacity

Type of Polyhead	Protein Components P23	P22	IPIII	IPII	In vitro Cleavage Capacity
20	+	+	+	+	+
$20+CHCl_3$	+	–	–	–	–
20-21	+	+	+	+	–
20-22	+	–	(–)	(–)	–
20-24	+	+	+	+	+
20-23 (E 920g)	+	+	(+)	(+)	+
20-IPO	+	+	–	–	+

The symbol (+) or (–) designates the presence or absence of a gene product, respectively. The parentheses indicate uncertainties. The amber mutations in genes 20, 21, 22, and 24 are (N50), (N90), (B270), and (N56), respectively. The hyphen designates a double mutant. The mutation (E 920g) in gene 23 is known to induce a large number of phage with shorter than normal heads (7, 8). The phage 20-IPO was kindly supplied by L. Black and designates a quadruple mutant in gene 20 and three internal proteins.

preformed τ-particles as these particles convert to the mature head (42). This demonstrates that these τ-particles from 24 defective cells contain little or no DNA and establishes that DNA is packaged into preformed, empty particles.

Genes Involved in the Maturation of the Head (Group B)

Genes in group B (2, 4, 13, 14, 16, 17, 49, 50, 64, and 65) are thought to be involved in the control of the maturation steps since infection of cells with phage carrying a mutation in one of these genes accumulate heads with at least the gross morphology of the normal wild-type head (44, 45). It needs to be pointed out, however, that the genes in group A discussed above may exert their function during the maturation steps. As an example, gene 24, which we classified in group A, appears to control the conversion of the prohead I particles into the prohead II particles (see below: Pathway – the head maturation steps).

The genes in group B are less well characterized than those in group A. It is noteworthy that so far none of their gene products have been identified by gel electrophoresis although our attempts have been quite extensive.

Genes 2, 50, 64, and 65. In 2, 50, 64, and 65 defective cells full and empty heads accumulate (44, 45), and a significant number of these heads have tails attached (45). The tail-containing particles found in 2 defective cells are able to kill bacteria but are unable to initiate a successful infection (45). It will be interesting to investigate the question whether or not these particles can inject their DNA into the bacterium.

Genes 2, 50, and 64 affect the cleavage of the various head proteins (17, 46). Cleavage of P23 to P23* occurs only to about half the normal extent, and the cleavage of P22 and IPIII appears to be similarly affected. P24, however, is cleaved to some extent in 2 and 64 defective cells but not at all in 50 defective cells. The particles isolated from 2, 50, and 64 defective cells contain, however, the cleaved head proteins (46), with the exception of the 50 particles which contain uncleaved P24 protein. So far we have been unsuccessful in isolating the particles containing the precursor proteins which are thought to exist in these cells.

Genes 16 and 17. In gene 16 and 17 defective cell heads accumulate which appear to contain no DNA (44) and are not associated with the replicative DNA (28, 48). Cleavage of the various head proteins occurs normally in these cells (17, 28) and the internal peptides are produced (49). We have speculated with little data that genes 16 and 17 may be involved in the attachment reaction of replicative DNA to the head (28) (see below: Gene assignment).

Gene 49. In 49 defective cells, packaging of the viral DNA into the head appears not to proceed to completion (28, 44, 50). The 49^- particles were reported to contain about 10–30% of the normal DNA content (50), but recently, using an improved lysis method, we have shown that these particles contain about 40–50% of the normal DNA (28), which is consistent with the electron microscopy studies of Granboulan et al. (45). The 49^- heads, which accumulate at nonpermissive temperatures in cells infected with the appropriate temperature-sensitive mutant phage, can be converted into active phage particles if the product of gene 49 is activated (50). Some unknown amount of DNA synthesized following activation of gene 49 can be packaged into the preformed heads (50).

Recently, we have carefully measured the amount of DNA incorporated into preformed 49^- particles as P49 is activated (42). We found that over half of the preformed particles incorporated 25 to 50% of the DNA complement, following activation of P49 (see below: Pathway – the head maturation steps).

The role of gene 49 in the DNA packaging event is not clear, but data of Frankel et al. (51) suggest that gene 49 codes for an endonuclease which cuts the large replicative DNA into head-size pieces in absence of any head formation. Therefore, it appears that some signal exists on the replicative DNA at head-size intervals which is recognized by this activity. Experiments by M. Curtis and B. Alberts (to be published) support this notion. They demonstrate that the large replicative DNA can also be converted to head-size DNA pieces in absence of any head formation in vivo. The production of head-size pieces commences in absence of any head formation if the product of gene 32 is inactivated in normally infected cells even if those cells contain a mutational block in a head gene. As predicted by Frankel, et al. (51), the active product of gene 49 is required for this conversion. Perhaps the product of gene 49 is needed to detach the DNA protruding from the head from the replicative DNA, which otherwise prevents completion of the packaging process by some kind of steric hindrance. In this case P49 need not be involved directly in the active packaging process.

Genes 13 and 14. In 13 and 14 defective cells full heads accumulate (44), which can be complemented in vitro (2). Subsequent studies showed that genes 13 and 14 control a terminal step of the head maturation prior to the attachment to the tail (70).

Pathway – The Head Maturation Steps

The data summarized in Fig. 1 do not represent a pathway of the assembly event, but illustrate the cooperation of the various genes in group A in the assembly of the various head-related structures.

One of the remarkable features of the T4 head assembly is the extensive cleavage of several head proteins which occurs during normal head formation. We have previously presented evidence (17) which suggested that cleavage of the various proteins occurs following assembly of the proteins into a large structure. The observation that the various head-related structures (Fig. 1) contain the precursor proteins is certainly compatible with this result. We have also speculated that the cleavage of head proteins could be the trigger

of the DNA packaging event (17). In order to determine at which point of the assembly cleavage occurs, we have designed experiments to elucidate the maturation steps of the head in detail (28).

The basic nature of the experiments which established this pathway are fractionation (on various gradients) of lysates of pulse-labeled wild-type and mutant-infected cells. The flow of the protein and/or DNA label through the various intermediates into the mature head is analyzed. The various intermediates are studied for their content of DNase-resistant DNA, their attachment to the replicative DNA, and their protein constituents (relative amount of precursor protein).

The major features of the pathway are the following: a) The viral DNA is pulled into an empty head in a series of events. b) Cleavage of two core proteins, P22 (MW = 31,000) to small fragments, and the internal protein IPIII (MW = 23,000) to IPIII* (MW = 21,000), appears to be intimately linked to the DNA packaging event, whereas the cleavage of the major head protein of the viral coat P23 (MW = 55,000) to P23* (MW = 45,000) precedes the DNA packaging event.

The prohead I particles. The prohead I particles are the first particles on the pathway. The genes listed to the left of the prohead 1 in Fig. 2 are required for the assembly of these particles. The phenotype and possible structural or assembly role of these genes were discussed above. The prohead I particles contain predominantly the precursor protein P23 and possibly the minor proteins P22 and IPIII. They sediment with about 400 S and are rapidly converted into the 350S prohead II particles. Most of P23 is cleaved to P23* during this conversion. The isolated prohead I particles appear to contain no DNA and are not attached to the replicative DNA.

The prohead I particles have properties similar to the so-called τ-particles isolated from 21 defective cells and 24 defective cells. The τ-particles isolated from 21 defective cells have a sedimentation coefficient of about 420 S and contain mostly the uncleaved protein P23, and the minor components P22, IPIII, and P20 (24, 38, 40). They appear to be bound to the inner membrane (1), and a detergent (Nonidet P40) is required to release these particles (21, 38). We find that this detergent treatment is also required for the isolation of the 400 S prohead I particles (28).

We think, therefore, that prohead I particles found in pulse-labeled wild-type infected cells are essentially identical to the τ-particles found in 24^- cells. Moreover, Simon (52) observed in thin sections of wild-type infected cells membrane-associated particles that had an appearance similar to the τ-particles and his studies also suggested that these particles are precursors to the normal head. The particles seen in wild-type infected cells as well as the τ-particles are filled with a core (25) (see below: The structure of the polyhead core).

Our experiment showed that the prohead I contains no DNA, but it could be argued that these isolated particles lose DNA during preparation. To remove this doubt we took advantage of the phenotype of temperature-sensitive mutants in gene 24 (see above: Genes 21 and 24). Recent experiments showed that τ-particles formed at nonpermissive temperature in cells infected with a ts mutant in gene 24 can be converted into active phage particles if the product of gene 24 (P24) is activated by temperature shift (41). It was therefore possible to examine whether DNA can be incorporated into preformed empty τ-particles as these particles are converted to mature heads.

We find that a very large amount (about 80%) of DNA made after activation of P24 is incorporated into the preformed 24-τ-particles as these particles are converted to the mature head (42). This shows that the 24-τ-particle indeed contains very little or no DNA

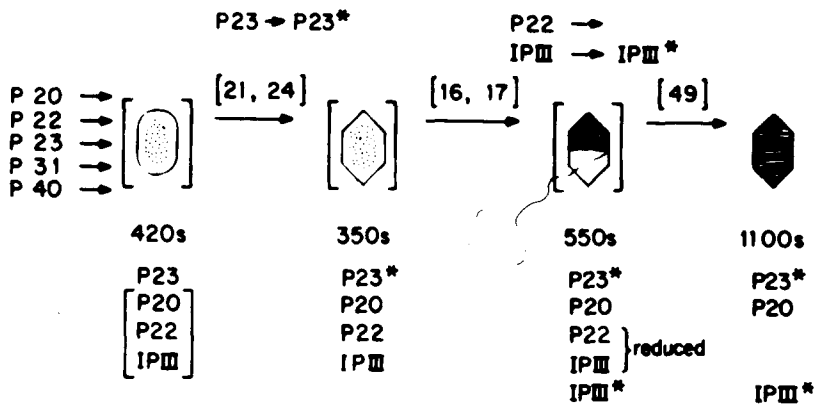


Fig. 2. Tentative pathway of head maturation. The major features of the maturation of the head are outlined. The numbers over the arrows indicate the genes which may be involved in controlling that step. The brackets indicate that the information is somewhat speculative and that we have no hard data for the assignment of these genes to a particular step. It is noteworthy to point out that cleavage is normal in 16^-, 17^-, and 49^- cells, whereas it appears from the above scheme that these mutants would be expected to block cleavage, at least of P22 and IPIII. The prefix P designates the product of a particular gene and the gene products found in each structure are listed. The list, however, is not complete.

and strongly supports the pathway shown in Fig. 2.

The prohead II. Most of the precursor protein P23 found in the prohead I particles is cleaved during the prohead I ⟶ II conversion and an S value change from 400 S to 350 S accompanies this conversion. The S value change is most likely due to the mass loss resulting from the cleavage reaction. The isolated prohead II particles contain no DNase-resistant DNA and are not attached to replicative DNA, but contain the core proteins P22 and IPIII.

The prohead III. The prohead III heads are the next particles on the pathway. They are filled with about half the normal DNA content, have an S value of about 550S and are attached to the replicative DNA. Cleavage of P22 to small fragments and IPIII to IPIII* occurs to roughly 50%. These particles have a very fragile DNA content. They are only observed if: a) the cells are lysed in presence of polyethylene glycol and high salt, conditions which are known to collapse DNA, or b) if the cells are lysed in the presence of glutaraldehyde (for more details of these methods see ref. 28). The conversion of prohead II to III is blocked in 16 or 17 defective cells, although cleavage occurs normally in these cells.

The mature head. The prohead III particle appears to be the immediate precursor of the full mature head (1,100S). Cleavage of P22 to small polypeptides and conversion of IPIII to IPIII* are complete at this time. No precursor proteins are observed in full heads. The mature head contains the internal peptides (15, 49), supposedly derived from P22 (27), and several proteins discussed above. The conversion of prohead III to the mature head is blocked by mutations in gene 49. Therefore particles accumulate in 49 defective cells which are about half full of DNA if isolated in the presence of polyethylene glycol or glutaraldehyde.

Again it is possible to argue that the isolated prohead III particles lose DNA during preparation (see Fig. 2). To remove this doubt, we have estimated the amount of DNA packaged into 49 defective heads when P49 is activated by temperature shift (42). The

uptake of BUdR into heads made at high temperature but matured in the presence of chloramphenicol and BUdR following shift to low temperature was studied. The 313 nm light survival curve as well as the buoyant density in CsCl of the phage matured in the presence of BUdR indicate that over half of the particles receive an average of 25% of the BUdR-DNA. This is a minimal estimate since the BUdR-DNA has to compete with unlabeled DNA. Analysis on alkaline sucrose gradients of the size of the DNA extracted from phage matured in the presence of BUdR following irradiation reveals that extended irradiation at 313 nm breaks the DNA to about 60% of its original size (42). These experiments clearly show that up to 50% of the DNA can be packaged after P49 activation into the preformed heads made at high temperature, and strongly support the pathway.

Gene assignment. It is not a straightforward task to assign genes to a particular step of the maturation process from in vivo data. A gene product may be incorporated into the structure at one point in the assembly pathway, but exert its function at a later point. Alternatively, a gene product may have a dual role, one exerted early and the other late in the pathway. Thirdly, the phenotypic expression of a mutant normally does not shed a clear light onto the step control by that gene, because the assembly may proceed in an alternative (possibly aberrant) route.

In order to assign a gene to a particular step unambiguously, it is necessary to be able to carry out this step in vitro, as is possible for many tail and tail fiber assembly steps (see e.g. ref. 47). We would like to emphasize for these reasons that the assignments made in the pathway presented (Fig. 2) are very tentative.

We have assigned genes 21 and 24 to control the prohead I to II conversion (28), simply because the available data suggest that the prohead I particles found in pulse-labeled wild-type cells have similar properties to the τ-particles. Recent data, which establish a precursor-product relationship between the τ-particles found in 24 defective cells and the normal heads (41), appear to warrant the assignment of gene 24 to this step.

We have also tentatively assigned genes 16 and 17 to control the prohead II to III conversion (28), since empty particles accumulate in 16 and 17 defective cells which are not attached to the replicative DNA. It is quite possible that this assignment is incorrect, since the argument is weak.

The conversion of the partially filled prohead III particles to mature heads does not occur in 49 defective cells and we have therefore assigned gene 49 to that step. This assignment may be warranted since this step can be blocked reversibly with temperature-sensitive mutants in gene 49, but it is possible that gene 49 controls this step only indirectly (see above: Gene 49).

RESULTS AND DISCUSSION

The Structural Components of the Polyhead Core

The aberrant tubular polyheads that accumulate in genes 20, 40, 22, and 24 defective cells are fragile and fall apart in lysates, and their purification requires a rapid isolation procedure. This procedure involves purification of the polyhead by differential centrifugation (22), followed by fractionation by sucrose gradient sedimentation (23). The polyheads sediment in a broad peak with an average sedimentation coefficient of about 400 S; the broad peak is due to the variable length of the polyheads. Polyheads have a length distribution between one and two microns in intact cells, but they are broken several times during the isolation procedure (29).

Previously only the precursor protein P23 had been identified in polyheads (36). A closer analysis, however, shows the presence of several minor proteins (23). In Fig. 3 the protein constituents of the polyheads isolated from cells lysed either by freeze-thawing or with the help of chloroform are compared on SDS gels. Both polyhead preparations contain the precursor protein P23 (Fig. 3). A small amount of the cleaved protein P23* is also observed in the polyheads prepared without chloroform but not in the polyheads prepared with chloroform. The significance of this will be discussed below. A major difference between the two polyhead preparations is observed in regard to their other protein constituents. The polyheads isolated from cells without chloroform contain the protein P22 and the internal proteins IPIII and IPII (Fig. 3a) whereas the polyheads from the chloroformed lysate lack these proteins (Fig. 3b). A broad band of unknown genetic origin just below P22 is also absent in polyheads isolated from the lysate treated with chloroform. No tail proteins are detected in the polyhead preparation, indicating that the polyheads are well purified. Positive genetic identification of the minor polyhead components was carried out by analyzing the constituents of polyheads isolated from various double mutant infected cells (23). Their compositions are summarized in Table I.

The quantity of proteins extracted from the polyhead by chloroform is substantial and for this reason we examined the two partially purified polyhead preparations in the electron microscope (23). We found that most of the polyheads prepared without chloroform appear to contain a proteinous core which is absent in the polyheads prepared with chloroform. From this and other data and the gel patterns discussed above, we have concluded that P23 is the protein constituent of the outer protein coat, whereas the proteins P22, IPIII, IPII, and supposedly IPI are the structural components of the proteinous core.

This observation is not unexpected. The internal proteins characterized by Black and Ahmad-Zadeh (16) reside on the inside of the mature phage head in association with the viral DNA (15). It is therefore reasonable to expect that these proteins would be in the interior of polyheads. Furthermore, Showe and Black (19) have suggested that P22 may be a core protein due to its association with the internal proteins.

The core proteins of the polyhead are organized into a highly ordered helical structure if the polyheads are isolated under appropriate conditions (26) (see below: The structure of the polyhead core).

In Vitro Cleavage of the Head Proteins of Partially Purified Polyheads

Cleavage of the various proteins occurs if partially purified polyheads are incubated at 37°C (23). We found that in a 100 minute incubation about 60–70% of P23 is converted to P23* and a significant conversion of IPIII (MW = 23,000) to IPIII* (MW = 21,000) is also seen. The protein P22 (MW = 31,000) disappears during this incubation and is supposedly cleaved to small fragments.

The in vitro products P23* and IPIII* have the same molecular weight as their respective in vivo products, suggesting that the cleavage is specific, but several protein fragments are generated during the in vitro cleavage reaction which have not been observed in vivo.

We examined a large number of compounds for their capacity to inhibit cleavage reaction and found that the most potent inhibitor is chloroform. Addition of chloroform to the reaction mixture inhibits the reaction immediately (23). (Cleavage is also inhibited if the cells are lysed with chloroform and incubation of the partially purified

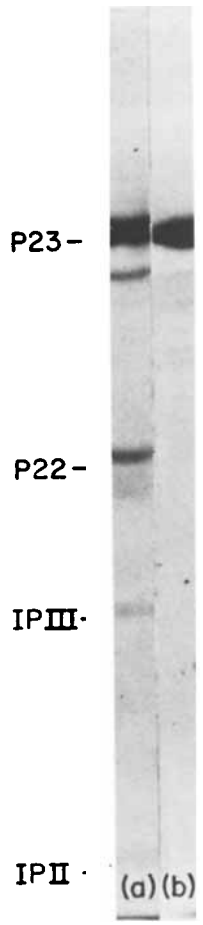


Fig. 3. Identification of the protein constituents of polyheads isolated from 20 defective cells on 12% acrylamide gels. [^{14}C]-leucine-labeled polyheads were isolated from 20 defective cells by sucrose gradient sedimentation as described elsewhere (23). a) Autoradiogram of the protein constituents of polyheads isolated from lysate lysed by freeze-thawing. b) Autoradiogram of the protein constituents of polyheads isolated from a lysate lysed with chloroform.

polyheads is done in the absence of chloroform.) In this context it is interesting to state that chloroform extracts the proteins P22, IPIII, and IPII from the polyheads (see above).

The polyheads used for these in vitro cleavage studies were isolated from 20 defective cells. Since the active products of many of the head genes (genes 21, 22, 24, 40, and the genes coding for the internal proteins) are not required for assembly of tubular polyheads (3, 5), it was possible to ask whether any of these genes code for a protein required for the in vitro cleavage reaction. We found that polyheads isolated from 20-24, 20-23 (E920), and 20-IPO defective cells cleave about as well as those isolated from 20 defective cells (Table I), whereas 20-21 or 20-22 polyheads do not cleave. Oronato and Showe [quoted in Showe and Black (19)] have also shown that a functional product of gene 21 is required for the in vitro cleavage of P22. Two alternatives are possible: either these genes (21 and 22) code for the proteolytic factor, or the absence of their product alters the substrate so that the proteolytic factor cannot function. The inhibitory

action of chloroform could be explained by assuming that the proteolytic activity is part of the core proteins and is inactive or diluted if extracted from the polyheads. This would also explain the observation that 20-22 polyheads cannot be cleaved. The multilayered polyheads found in those cells fall apart into individual tubes containing no core proteins (23) (Table I). Thus, genetic as well as chemical elimination of the core proteins prevents the cleavage reaction. This observation suggests that the proteolytic activity is part of the core of polyheads, but does by no means establish this point. Note that the partially purified polyheads are still heavily contaminated by other phage and bacterial proteins. Attempts to cleave highly purified polyheads isolated by sucrose gradient sedimentation have failed, so it is possible that the proteolytic activity is purified away from the polyheads. On the other hand, we observed that these polyheads fall apart and thus may become an unsuitable substrate.

It has been suggested that P22 is the precursor of the internal peptides (27). This finding is consistent with the observation of Sternberg and Champe (21) that the genetic determinant of internal peptide II resides in the 20 to 22 gene region of the phage T4 genome. Recently, Goldstein and Champe (53) have also described an in vitro cleavage system different from ours. They observed that internal peptide II can be produced in the in vitro cleavage reaction and that the formation of the peptide in vitro is dependent on a factor found in extracts of phage-infected cells but absent from extracts of uninfected cells. We do not know whether our cleavage reaction produces the internal peptides.

The Structure of the Polyhead Core

The surface structure of the polyhead has been studied extensively. It consists of a hexagonal arrangement of capsomers, which can be resolved into six structural units made up of the protein P23 (30–33). Little information has been available about the organization of the core of the polyhead. This is due to the lability of the core, which breaks down into amorphous clumps of material or falls out of the polyhead completely when polyheads are isolated by conventional means and viewed by negative staining in the electron microscope. Some structure of low resolution is seen in the inside of polyheads examined in thin sections of cells (25).

Information on the spatial organization of the core is of great importance since we know that a) the protein P22 is involved in the initiation and size determination of the head (3) and b) cleavage of the core protein P22 appears to be linked to the DNA packaging process (28).

We have developed a lysis procedure which permits the isolation of polyheads with highly organized cores (26). This procedure involves lysis of spheroplasted cells into a buffer containing glutaraldehyde (28). Electron micrographs of such polyheads are shown in Fig. 4b, c, and d; they are to be compared with the polyheads isolated without glutaraldehyde (Fig. 4a). It is apparent that the polyheads isolated with the fixative are filled with a core and, for this reason, do not flatten down as do the polyheads isolated without the fixative. The core of the fixed polyheads appears to be highly ordered since a "herringbone" or cross-hatched structure can be seen (indicated by the arrows in Fig. 4c and d). As a first approach to solve this structure, we have combined optical diffraction with the optical superposition method of Markham et al. (54). In the latter method an improved image is produced by superimposing photographically several copies of the original image translated by what is thought to be the basic repeat of the structure. Features related to that repeat will be preserved and enhanced, while features of a

different repeat will be smeared out and thereby suppressed.

Of course, the basic repeat needs to be established. One way of doing this is to measure directly the repeating features in good areas of the electron micrographs. However, this is unsatisfactory since these features are rarely clear enough to be unambiguously and accurately measured. The better technique is to study the optical transform of the structure; so we have analyzed a large number of optical transforms of core-containing polyheads.

Many of these optical transforms turned out to be very noisy. But in a few cases we were able to identify reflections from the hexagonal surface lattice as well as reflections which are due to the core. We identified the surface lattice by comparing these transforms with those of empty polyheads. The size of the unit cell was found to be about 110 Å for the surface lattice, in agreement with the reported lattice length of empty polyheads (33).

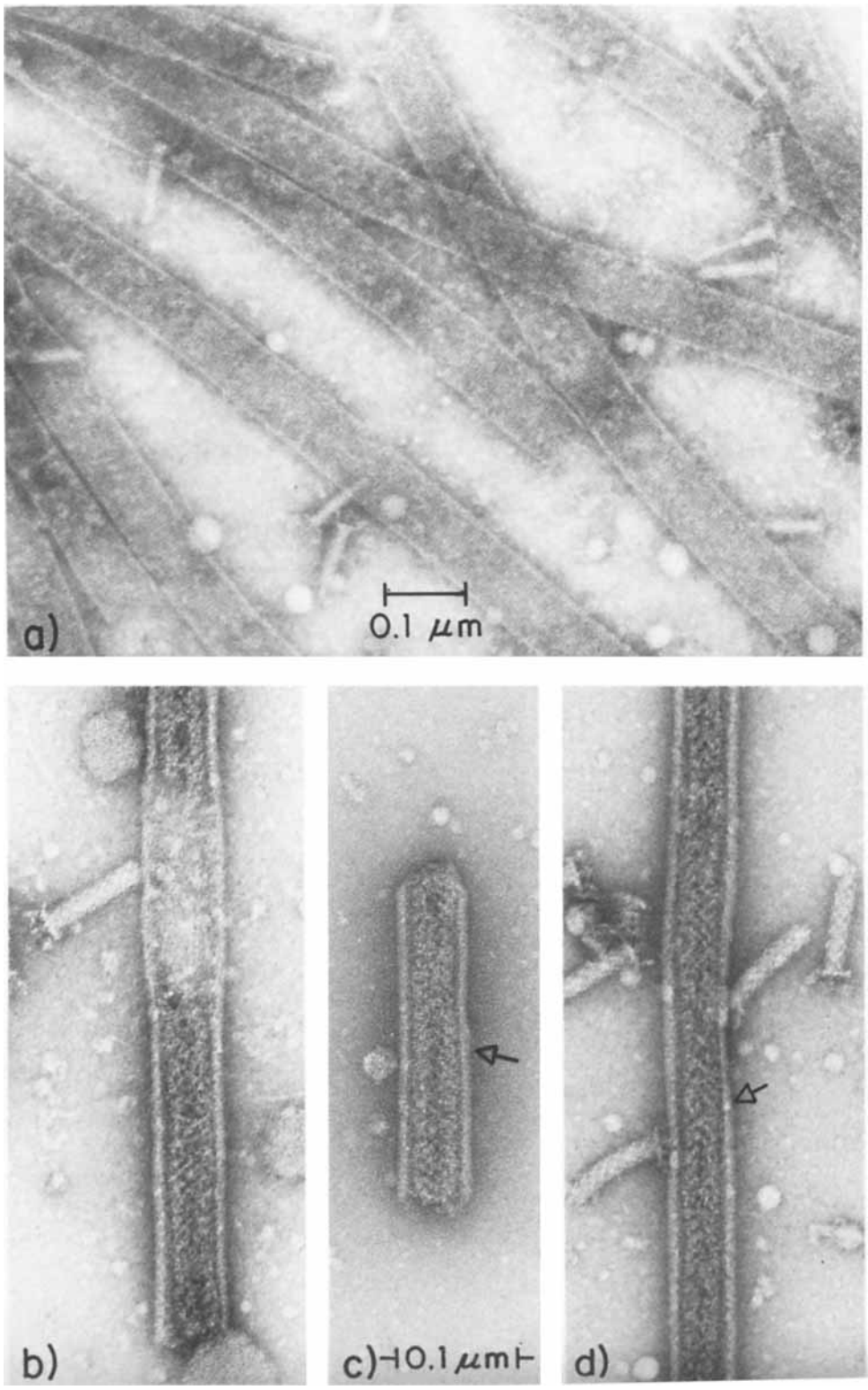
Core-containing polyheads show an additional feature (not seen in empty polyheads) in the form of four strong reflections at an axial spacing of about 137 Å, arranged symmetrically – roughly in a square – in a pattern characteristic of a helical structure.

In order to verify this interpretation of the optical transform and to obtain an improved picture of the polyhead core, we used the optical superposition method.

Figure 5a shows the original image. The polyhead on the right was studied. The most prominent features of the polyhead surface are the striations spaced 55 Å apart seen along the edge of the particle. (We do not expect to see surface features over the center of the particle because the contrast provided by the surface may be obliterated by the much more heavily stained core.) We expect that a superposition image produced by translating by the surface repeat should enhance these surface features. Indeed, in Fig. 5b the superposition was performed in increments corresponding to 110 Å, and the repeats along the edge are enhanced. The core has been partly suppressed – it does not show up as clearly as in Fig. 5c (see below) – but not completely. This is because of the small number of superpositions used in making the image and because of the high contrast in the core.

Similarly, an optical superposition image produced by translation of the original in the axial direction by the core repeat of 137 Å should enhance the structure of the core. This is shown in Fig. 5c. It is evident that the core consists of several helices spaced about 137 Å apart. The repetitious features along the edge, however, are smeared out, showing that the surface does not have this periodicity. The image of the core varies in its two-sidedness; in some places stain has contrasted both the near and far sides, giving a cross-hatched appearance to the core, whereas in other places the image of one side predominates. The helices appear to be wound about a hollow center of approximately 150 Å diameter. We have tried to determine the number of helically wound chains in the core structure, but the calculation is subject to uncertainty because we cannot determine accurately the dimensions of the core. Our best estimates indicate that there are between 4 and 6 helically wound chains.

We have not identified any higher order reflections due to the core, and therefore we have no information about the subunit structure of the helices.


a)
0.1 μm
b)
c)
0.1 μm
d)

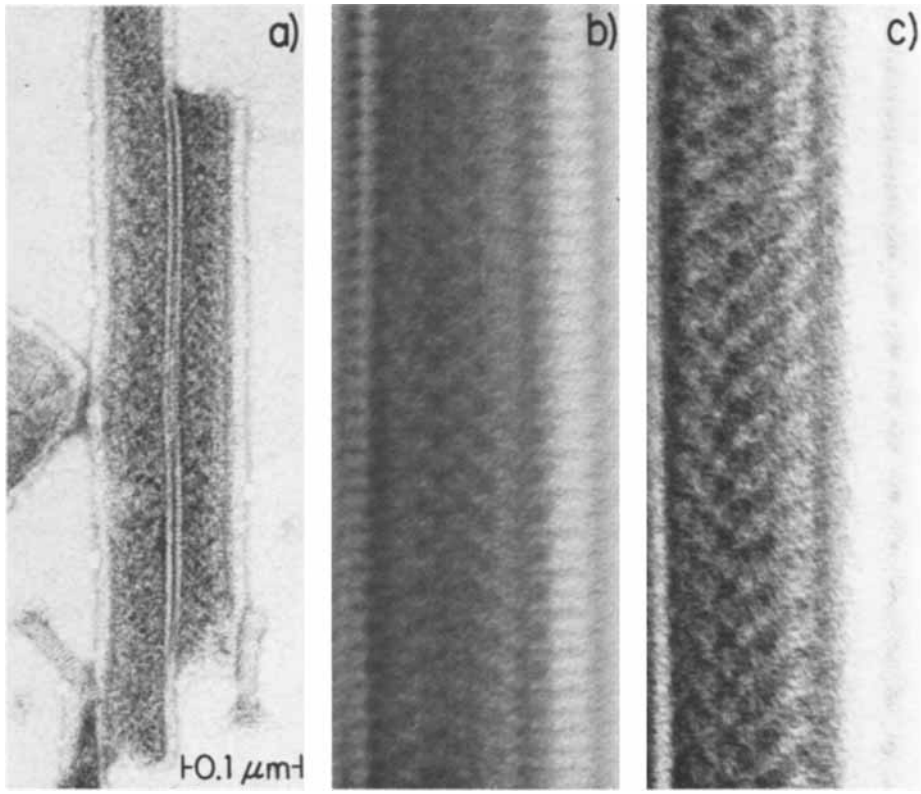


Fig. 5. Superposition image of core-containing polyheads. a) Polyheads prepared as described in Fig. 4. The polyhead on the right was used to make the superimposed polyhead images in b) and c). Note the cross-hatched or "herringbone" structure in the core and the striations along the edge of the particle. In b) the image was translated by a distance corresponding to 110 Å and superimposed 10 times. In c) the image was translated by a distance corresponding to 137 Å and superimposed 6 times.

Fig. 4. Electron micrograph of core-containing polyheads. a) Polyheads without cores isolated by the method of Laemmli and Quittner (23). b), c), d): Polyheads with cores isolated with glutaraldehyde by the method of Laemmli and Favre (28). In b) note the region where the core has begun to fall out. The glutaraldehyde-fixed polyheads were prepared as follows: 20 ml cultures of E. coli B^b in M9 medium were grown at 37° C to $2-3 \times 10^8$ cells/ml and then infected with the mutant 20 (am N50) or the double mutant 20 (am N50) – e (am H26) at a multiplicity of 5, and superinfected 8 min later. L-tryptophan was added one minute before infection to a final concentration of 2 μg/ml to enhance phage adsorption. Forty minutes after infection cells were chilled rapidly and concentrated by centrifugation for 10 min at 3000 g. Pellets were resuspended in 2 ml of 25% sucrose in neutral phosphate buffer with 10^{-3} M EDTA and 50 μl of T4 lysozyme (1 mg/ml) and incubated on ice for 30 minutes. Cells were lysed by addition of 8 ml cold neutral phosphate buffer containing 10^{-3} M $MgSO_4$. One hundred μl of freshly opened 8% glutaraldehyde (Polyscience) was added immediately, and the sample was agitated briefly and placed in a waterbath at 37°C. One hundred μl of 11% ethylamine solution was added after 5 min followed by a 10 min incubation to remove excess glutaraldehyde. Then 100 μl DNase (2 mg/ml) was added for a further 10 min incubation. Polyheads were partially purified by differential centrifugation and resuspended in 0.3 ml phosphate buffer with 2 mM $MgSO_4$. Samples were applied for 1 min to formvar and carboncoated grids, rinsed with distilled water, and stained with 1 or 2% uranyl acetate, and finally photographed at an original magnification of about 50,000 in a Phillips 300 Electron Microscope.

Support for a Possible DNA Packaging Mechanism

How could the DNA be pulled into a preformed empty head and how could cleavage of P22 be linked to the DNA packaging process? All the models to be discussed will be based upon the assumption that the DNA becomes fixed to a protein bound to the inside of the head. In the model we will propose the packaging process is initiated by the formation of a local environment inside the head which collapses DNA. As the DNA collapses in the interior, it will exert a pulling action on the external part of the DNA since the end is firmly attached inside the head, and so the rest of a head-sized piece will be drawn into the head. The attractive feature of this model is that DNA is pulled into the head as a consequence of the collapse of the DNA inside the head.

As an approach, we have initiated experiments to test whether any of the head proteins or peptides are able to collapse DNA. Before we describe the evidence for the working hypothesis that we favor, we would like to enumerate possible ways by which DNA can be collapsed: a) *Dehydration.* Lang (55, 56) has shown that T7 DNA collapses into tight rodlike structures visible in the electron microscope if ethanol is added to the DNA solution. His experiment suggests that the DNA is collapsed into super-coils of different order depending on the ethanol concentration used. b) *Condensation at low pH.* DNA aggregates below pH 2.5 (57). Light-scattering data suggest that these aggregates are fairly compact with an effective diameter of 0.15–0.3μm for T4 DNA (58). Electron microscopy data confirm this but do not reveal the structure of the collapsed DNA (59). c) *Titration with a protein.* DNA can be collapsed by titration with polylysine. This collapse occurs only if all phosphate groups of the DNA are titrated by the polylysine (60). The structure of polylysine-collapsed DNA has been studied and will be discussed below. d) *Interaction with a DNA-binding protein.* Alternatively, it is possible that the DNA is collapsed not by complete titration with a basic protein, but by interacting with a DNA-binding protein. Sequential production of such DNA binding sites inside the head could wind up the DNA (17). We will call this model the "DNA-binding site model." e) *DNA collapse by repulsive interaction.* DNA can be condensed by repulsive interactions. Lerman and his collaborators (61, 63) have demonstrated that in a salt solution containing a sufficient concentration of a simple polymer like polyethylene oxide or polyacrylate, high molecular weight DNA undergoes a cooperative structural transition which results in a very compact configuration. The salt is required for charge neutralization of the DNA. Lerman's circular dichroism, x-ray, and birefringence studies show that the collapsed DNA is highly ordered and compact (62, 63).

It is conceivable that the phage employs one or a combination of these mechanisms to package the DNA into the preformed head. The first two mechanisms, dehydration and acid precipitations, are unlikely candidates for obvious reasons. If the DNA is collapsed by titration or by the attachment of the DNA to specific sites (DNA-binding site model), then one would expect to find structural head proteins which have the capacity to bind to DNA. The internal proteins are known to bind to DNA (64), and we have extensively characterized their DNA-binding capacity (65), but recent experiments by Black (18) demonstrate that the internal proteins are nonessential proteins of the phage head. This appears to eliminate their possible role in the DNA packaging process (see above: The internal protein genes). However, other DNA-binding head proteins do exist and will be discussed below.

In the following we will present evidence which suggests that a repulsive interaction between the internal peptides and the DNA may collapse the DNA inside the phage head.

We have shown that cleavage of P22 appears to be associated with the DNA packaging process (28). P22 is cleaved to small fragments (17) and it had been suggested that P22 is the precursor of the internal peptides II and VII (19). More recent evidence shows that at least peptide II is quite definitely derived from P22 (27). These internal peptides were first described by Hershey (15) and are known to be derived from a larger precursor protein (49). An extraordinary characteristic of these peptides is their amino acid composition. Both peptides are highly acidic (66); 80% of the amino acid residues are glutamic or aspartic acid for peptide II and 48% for peptide VII. Although they do not bind to DNA (65), their highly acidic nature led us to think (28) that they might collapse DNA by a repulsive interaction in much the same way as polyacrylate. Support for such a possibility will now be presented. The experiments will be published in greater detail elsewhere. Since the internal peptides are highly acidic, we have tested whether model compounds like polyaspartic acid and polyglutamic acid can collapse DNA. Increasing amounts of the polymer are added to a salt solution containing radioactive T4 DNA. When a critical polymer concentration is reached, large DNA aggregates (61) form and can be collected by low speed centrifugation. Figure 6 shows that most of the DNA is removed from the supernatant by polyacrylate above a concentration of about 80 mg/ml. Polyglutamic acid of a molecular weight of 70,000 daltons also collapses DNA above a concentration of about 150 mg/ml, whereas more than 300 mg/ml of polyaspartic acid of a lower molecular weight, 4,300 daltons, is required to collapse the DNA. Clearly, the method used to determine the critical concentration for DNA collapse is crude, but these experiments show that DNA does collapse if sufficient amounts of polyglutamic or polyaspartic acid are added. We have also purified a large amount of the internal peptides as described by Eddleman and Champe (49) and tested these peptides for their capacity to collapse DNA. The results in Table II show that most of the DNA is removed from the solution somewhere in the peptide concentration range of 100 to 500 mg/ml. Because it is necessary to use a large amount of internal peptides in this experiment, a more accurate determination of the critical concentration was not possible. Nevertheless, it is clear that high concentrations of the internal peptides do collapse DNA.

Of course, these experiments provide no information about the structure of the collapsed DNA. It is reasonable to argue, however, that the structure of DNA collapsed with polyglutamic acid, polyaspartic acid, or the internal peptides is similar to that of DNA collapsed with polyethylene oxide (PeO) or polyacrylate (PAA). Lerman (61) demonstrated that PeO or PAA collapses T4 DNA into particles approaching the compactness of the contents of phage heads. We have started to study the structure of PeO-collapsed DNA in the electron microscope in order to compare this structure with that of DNA inside the phage head. If, indeed, the DNA is collapsed by a repulsive interaction, then their structures should be similar. Included in this study was DNA collapsed by titration with polylysine in order to contrast these different methods.

Olins and Olins (67) showed that DNA collapsed with polylysine appears in the same preparations as either donuts or short stem structures. No substructure was visible, however, in their electron micrographs. Our studies confirm this, but we used negative staining and a different method to adsorb the DNA to the electron microscope grid. This method permits visualization of the DNA strand in the folded complexes (see Fig. 7).

Figures 7 and 8 show the donut and stemlike structure of polylysine-collapsed DNA. The DNA, visible as a 25 Å repeating striation, is closely packed in the stem structure and is probably folded back and forth in a pleated structure, since the total extended length of the DNA (T7 or T4) is many times longer than that of the stem. There is also some

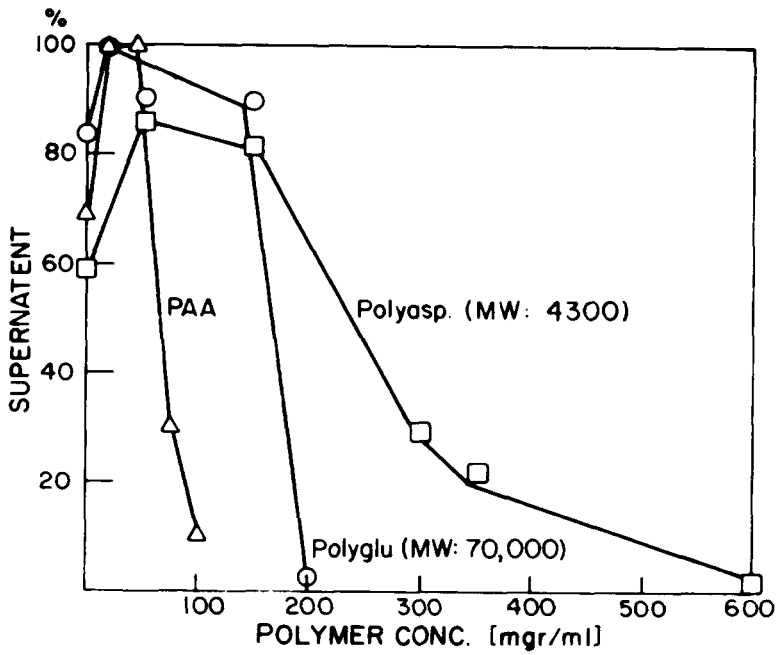


Fig. 6. DNA collapsed with various acidic polymers. Polyacrylate (Colloids), polyglutamic acid (Pilot), and polyaspartic acid (Miles) were dissolved in neutral phosphate buffer (50 mM) containing 1 M of NaCl and 10^{-3} M of EDTA by adjusting the pH to neutrality with the addition of concentrated NaOH. Fifty ml of the polymer solution of various concentrations were carefully mixed with an equal volume of [^{3}H]-labeled, phenol-extracted T4 DNA of a concentration of 20–30 μg/ml. The samples were slowly rotated at room temperature for 45 min. The samples were centrifuged at 4°C in SW50.1 at 16,000 rpm for 45 min. The acid precipitable counts were determined from aliquots of the supernatant and the results are expressed as the percentage of the total DNA remaining in the supernatant. About 30% of the total DNA appears to be aggregated and sediments without any polymer added. Note, however, that the addition of a small amount of polymer appears to dissociate these aggregates since the amount of DNA in supernatant rises initially.

indication of these folds in the fine structures of these stems. The DNA appears to be radially distributed as a spiral in the donut structures. These two types of structures are about equally frequent in any single preparation. We can roughly estimate the number of layers in thickness of both structures as follows: The number of DNA strands across a stem can be counted in the best areas of the electron micrographs (about 13 in Fig. 7c), and taking into account the total length of the linear DNA, we can estimate the expected number of layers of DNA. Our estimate establishes that both donuts and stem structures are about 3 to 4 layers thick, assuming close packing of the DNA strands.

The structure of the T4 DNA collapsed with PeO is different. In the electron microscope, ellipsoid particles are seen (Fig. 9). The average length (20 particles measured) is about 1,000 Å and their width about 600 Å. These particles are therefore about the size of the T4 head. Phage heads present in the preparation were also used for a direct comparison.

The suborganization of the DNA is more difficult to see, but a 25 Å striation tangential to the particle is easily recognizable. These particles may be best described as

TABLE II. DNA Collapsed with the Acidic Internal Peptides

Peptide Concentration mg/ml	% DNA in Supernatant
0	75
20	97
100	40
500	3

27 mg of internal peptides purified as described by Eddleman and Champe (49) were dissolved in 50 μl of neutral phosphate buffer (50 mM) containing 1 M of NaCl and 10^{-3} M EDTA. Five μl of [^{3}H] -labeled, phenol-extracted to T4 DNA at a concentration of 15 μg/ml was added carefully to 25 μl of internal peptide solution of various concentrations and mixed by rotation for 45 min at room temperature. The fraction of DNA in the supernatant following centrifugation was determined as described in the legend to Fig. 6.

a ball of string. The DNA strand is most clearly visible in particles which appear to be less compact (see arrow, Fig. 9).

These particles must be quite thick, since the surface area (assuming close packing of the DNA) only accommodates about 5% of the DNA; one can calculate that they are 400–500 Å thick. The electron micrographs, however, do not reveal the orientation of the DNA in the different layers. It is possible that suborganization of the DNA strands in the PeO-collapsed DNA is very similar to that of the polylysine-collapsed DNA, although it is clear that the overall shape of the DNA is different in these two cases.

These electron microscopy studies compare favorably with Lerman's sedimentation analysis of PeO-collapsed DNA. T4 DNA collapsed with PeO has a sedimentation coefficient between 300 and 700 S, and they calculated that the faster species approach the compactness of DNA in the phage head.

Recently, Richards et al. (68) have obtained electron micrographs of several coliphages which indicate the way the DNA is arranged within the head. They showed that the DNA appears as a set of concentric circles or as a tightly wound spiral. Two models were proposed: in one, the DNA is packaged like a ball of string; whereas in the other, it is wound coaxially like a spool. Our electron micrographs of DNA collapsed with PeO are compatible with both models. (Note, however, that the PeO-collapsed particles are differently oriented on the electron microscope grid). The observation that PeO collapses DNA into a structure similar to that of the in vivo DNA condensate supports the view that a repulsive interaction may be involved in packaging the DNA within the phage head but does not rule out a collapse caused by a basic protein like polylysine.

Is such a model reasonable? The internal peptide concentration needed to collapse DNA in vitro is between 100 and 500 mg and we will assume for the following discussion that about 400 mg/ml is required. We also assume that both internal peptides II and VII are derived from P22, although this has not been rigorously established. The concentration of internal peptide within the head, assuming that they have access to the total head volume and assuming that the internal peptides make up about 0.4% of the total phage mass (66), is about 40 mg/ml. It appears therefore that the internal peptide concentration is too low. But there are three variables which we have not considered: a) It is possible that the internal peptides produced by cleavage remain confined to a small area within

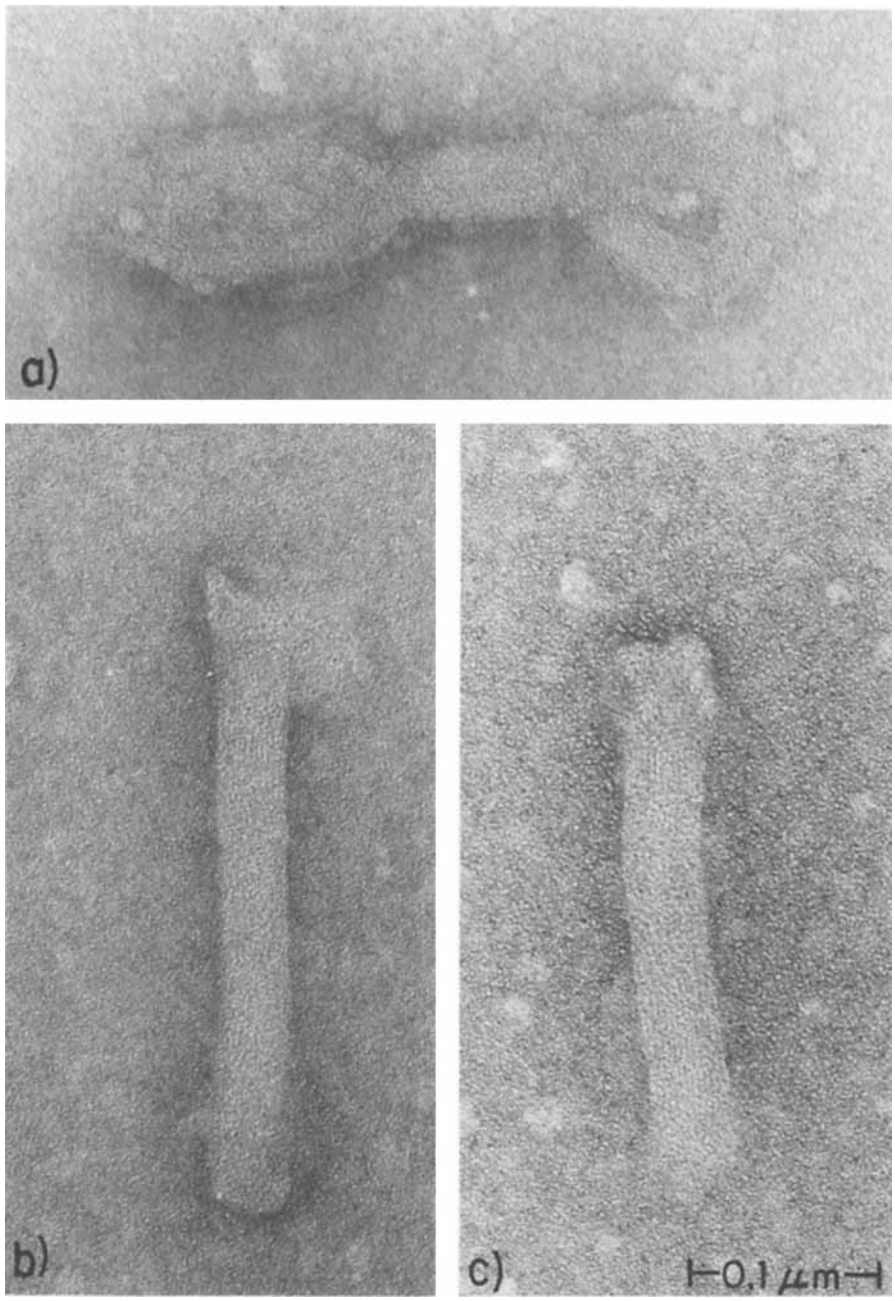


Fig. 7. Stem structures seen in preparations of polylysine-collapsed DNA. Poly-D-lysine of a molecular weight of 40,000 was dissolved in neutral phosphate buffer (50 mM) containing 1 M of NaCl and 10^{-3} M EDTA to a final concentration of 0.1 mg/ml. T4 DNA prepared as described in Fig. 9 and phenol-extracted T7 DNA, both at concentrations of about 0.5 μg/ml, were added to the poly-D-lysine solution by mixing equal volumes slowly by rotation.

Formvar, carbon-coated grids were pretreated with poly-D-lysine (MW = 40,000) as follows: a drop of 1 μg of poly-D-lysine solution in water was displayed on a parafilm and formvar, carbon-coated grids placed on the droplet for 30 sec to 1 min. Excess liquid was removed with a filter paper and the grids air-dried. The grids were rendered hydrophilic by glow discharge.

The poly-D-lysine-coated grids were used to pick up the collapsed or linear DNA. A drop of DNA solution collapsed as described above was displayed on parafilm and a poly-D-lysine-coated grid was placed on the droplet for 30 to 45 min. The grids were washed with water by repeated touching to a water surface and negatively stained with 2% uranyl acetate. So far no experiments have been made to compare the poly-D-lysine-coated grids with untreated grids.

Panels b) and c) show stem structures of T7-collapsed DNA and panel a) shows an unusual, rarely observed structure of poly-D-lysine-collapsed T4 DNA.

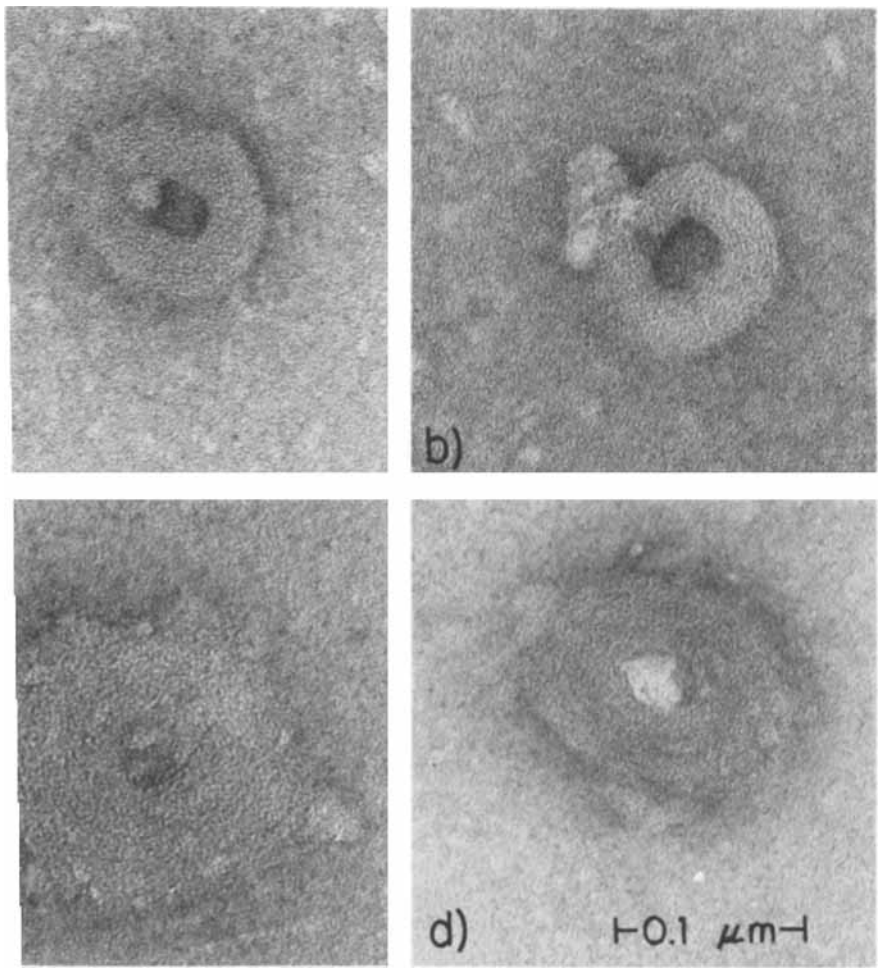


Fig. 8. Donut structures seen in preparations of poly-D-lysine-collapsed DNA. The donut structures presented here are observed in the same preparation as described in Fig. 7.

Panels a) and b) show poly-D-lysine-collapsed phage T7 DNA and panels c) and d) show poly-D-lysine-collapsed T4 DNA.

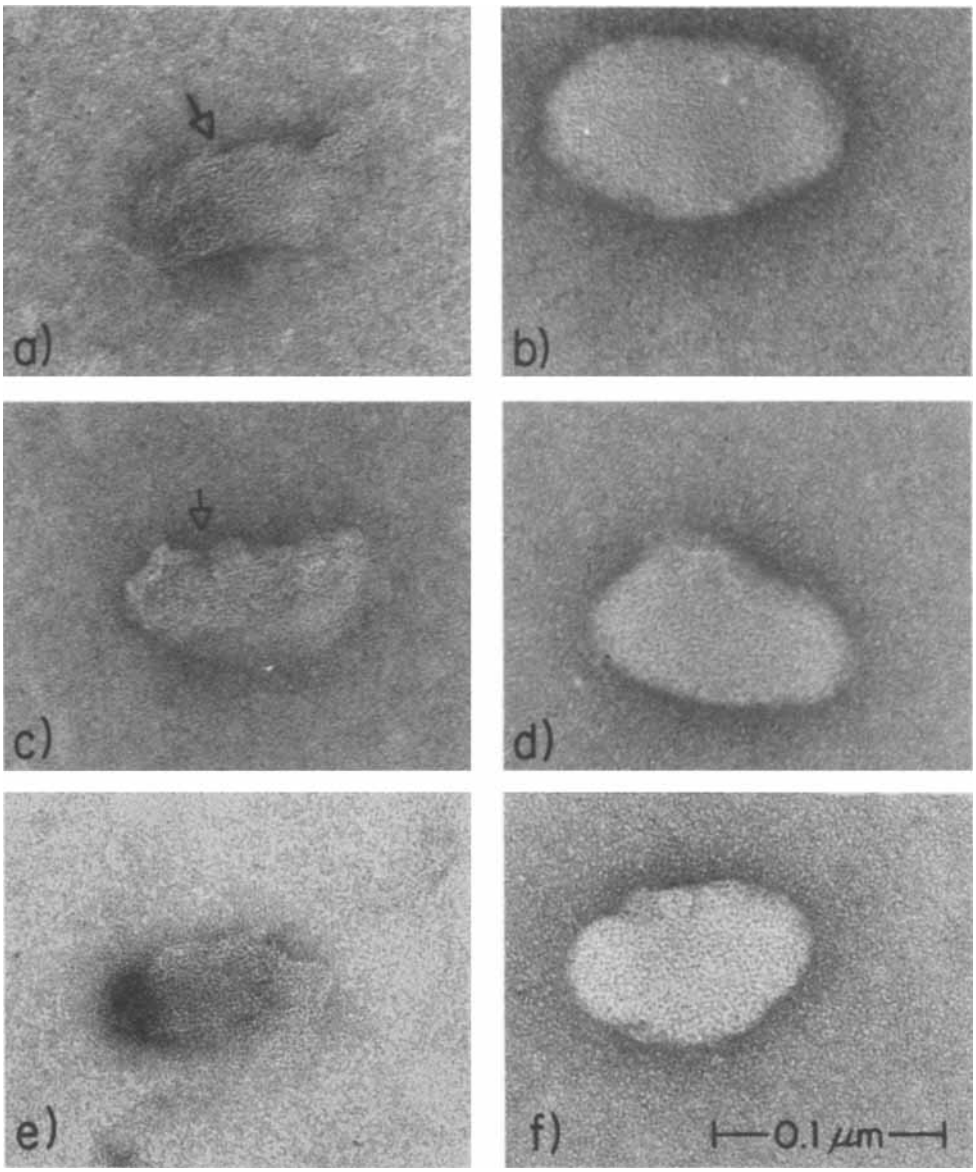


Fig. 9. Particles of polyethylene oxide-collapsed DNA. T4 DNA prepared as described below was diluted into neutral phosphate buffer (50 mM) containing 0.5 M NaCl, 10^{-3} M EDTA and 100 mg/ml of PeO (6000) and kept at 4° C overnight. The collapsed DNA was applied to a poly-D-lysine-coated grid as described in the legend to Fig. 7. The DNA was prepared by dialysis of partially purified tailless heads against a neutral phosphate buffer (50 mM) containing 0.5 M NaCl and 10^{-2} M EDTA overnight. This procedure releases the DNA gently from the heads. Tailless heads were prepared from a 200 ml culture infected as described elsewhere (28) with a double mutant amB255 and amE18 in genes 10 and 18. The cells were concentrated 50 min after the infection by low speed centrifugation and resuspended in 2 ml of neutral phosphate buffer containing 2 mM of $MgSO_4$ and lysed with chloroform. Twenty μl of pancreatic DNase (2 mg/ml) was added and the sample incubated at 10 min at 37°C. Bacterial debris was removed by centrifugation for 10 min at 5000 g, and the heads in the resulting supernatant concentrated by centrifugation for 30 min at 30,000 g. The pellet was gently resuspended in 2 ml of neutral phosphate buffer containing 2 mM of $MgSO_4$. The sample was subjected to another centrifugation for 10 min at 5000 g and then dialyzed as described above to release the DNA from the phage heads.

Panels a), c), and e) show PeO-collapsed T4 DNA. These particles are somewhat smaller than the average particles and appear less compact, but the DNA strand is more clearly visible.

Panels b), d), and f) show representative particles of PeO-collapsed T4 DNA.

the head. As the DNA is collapsed in these confined areas, it takes up volume, and this could maintain or even increase the effective peptide concentration. We should point out that the DNA in the mature head takes up about 80% of the available head volume. Thus, assuming that the internal peptides are confined to a small area of the head and taking into account the volume taken up by the DNA, it is conceivable that the internal peptide concentration is sufficiently high to drive the DNA packaging event. b) We have assumed that the internal peptides alone are involved in the repulsive interactions. The combined molecular weight of the peptides is about 6,400 daltons (49, 66) while their precursor P22 has a molecular weight of 31,000 (17, 20). Thus about 80% of P22, which is also highly acidic (27), is not accounted for and is probably cleaved to very small peptides or amino acids. Although this part is not found in the mature phage, it may function during the DNA packaging events. Including this part of P22, the acidic peptide concentration would be about 250 mg/ml. It is also noteworthy that the critical concentration required to collapse DNA diminishes with increasing molecular weight of the collapsing polymer. c) It is possible that the piece cleaved off P23 during its conversion to P23* is also involved in the DNA packaging process. This fragment (expected MW = 10,000) is also not found in the mature phage, but may exist in the head during the maturation process. It is also quite acidic as deduced by comparing the amino acid composition of P23 to that of P23* (27). If this fragment is included in the calculation of the acidic peptide concentration, a value of over 500 mg/ml is obtained.

Although this discussion is based on many assumptions, it does suggest that the concentration of acidic peptides may be high enough to drive the DNA into a collapsed form. In the model, a DNA binding protein would be involved in the initial attachment of the DNA to the inside of the head, but no further DNA: protein interactions are proposed. We have found two head proteins which need to be characterized further that have high affinity for DNA (65).

In considering mechanisms for the DNA packaging process, one has to take into account that the DNA has to be injected into the bacterium during the infection process. The DNA must therefore be collapsed reversibly. Moreover, it is likely that a positive driving force is required to inject the DNA through the tail and into the bacterium. In the context of the model discussed, one could propose that the concentration of acidic peptides is lowered below the critical concentration once the DNA is successfully packaged. For example, the acidic peptide concentration may be lowered if the cleaved fractions of P22 and/or P23 are involved in collapsing the DNA but after packaging are further hydrolyzed to amino acids or peptides small enough to leak out of the head. The DNA in the mature phage would thus be in a constrained configuration, and, upon reinfection, would be rapidly injected through the tail. Another possibility is that the concentration of peptides II and VII is sufficient to stabilize the collapsed DNA in the mature head, but that upon reinfection the acidic peptides leak out through the tail and the DNA configuration becomes unstable. The destabilized DNA would then, by self repulsion, rapidly inject into the bacterium.

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