DNA Packaging in dsDNA Phages

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INTRODUCTION

Imagine trying to stuff a string more than six micrometers long into a sphere that is fifty nanometers in diameter. The hole in the sphere that the string must enter is only twice as wide as the string itself. The string is stiff, with a persistence length on the order of one hundred nanometers. It is also negatively charged and self-repulsive. The string must be organized such that it can be pulled out easily, so no knots or tangles are permitted. When the sphere is full, the string will have a near crystalline density. You have several minutes to complete this task. This difficult feat is the challenge presented to dsDNA phages during DNA packaging, a pivotal event in the assembly cascade.

The task of compacting the double-stranded DNA chromosome into a protein capsid is a dramatic endeavor. DNA by its nature does not want to be in condensed form, but rather is dispersed, occupying a volume more than 100 times its volume inside the virion (Hohn, 1976; Kellenberger *et al*., 1986). Therefore, in order to be packaged, energy must be invested in the DNA. The DNA packaging event must also be coordinated with the replication of the phage DNA that is to be packaged, as well as the assembly and maturation of the protein capsid. Numerous investigators, using a battery of model phage systems, have made a concerted effort over four decades to resolve the components and mechanism of DNA packaging.

Descriptions of the specific components and processes involved in DNA packaging for many of the phages are described in the accompanying chapters of this book. Our intention here is to describe the specific challenges of double-stranded DNA packaging in bacteriophages and detail the common events and structures involved. For

most of the systems dealt with here, an extensive battery of biochemical and genetic resources has accumulated over the past half century. Defined *in vitro* DNA packaging systems have been developed for many of the phages we will describe (T3 in Hamada *et al.*, 1986; T7 in Son *et al.*, 1989; T4 in Rao and Black, 1985; λ in Hwang and Feiss, 1995; ø29 in Guo *et al.*, 1986). This ability to manipulate DNA packaging has been the hardy complement to the genetic, biochemical and microscopy approaches that preceded, and now parallel, the development of these experimental systems. More recently, structural data has come to the forefront of efforts to understand DNA packaging in the form of cryo-electron microscopic reconstruction of phage structures and X-ray crystallographic and NMR analyses of components of the DNA packaging machine. These advances bring additional relevance to the study of DNA packaging in bacteriophages and offer the opportunity to elucidate the mechanism of DNA packaging.

COMPONENTS OF DNA PACKAGING SYSTEMS

In order to provide an informative account of the phage DNA packaging process, we will first briefly review the components involved in packaging in some well-characterized phage systems. All of these phages have a double-stranded DNA to be packaged; a prohead receptacle for the packaged DNA; and packaging ATPases, enzymes that procure the DNA substrate and mediate the conversion of chemical energy to mechanical energy required to translocate the DNA into the prohead. The convergence of the maturation pathways and the interaction of these components comprises the DNA packaging event.

DNA

The phage DNA chromosome must retain the information to do three things: ensure its own replication to produce chromosomes to be encapsulated into progeny virions; commandeer the host cell metabolism and redirect it toward the production of progeny virions; and encode the structural proteins and enzymes required to assemble new virions. To achieve the first goal, a number of strategies yield forms of replicated DNA that are presented as immature chromosomes to be packaged. Virion DNA of the dsDNA phages is linear and is packaged processively, generally from left to right with respect to the conventional genetic map (an exception is the T3/T7 systems, which package right to left). There is a teleonomic relationship between the DNA replication strategy of a given phage and the form of the linear DNA encapsulated in the virion. The key to the relationship lies in the replication of a linear DNA molecule upon infection without loss of genetic information needed to prime DNA synthesis at the 5' end. DNA replication strategies and the resulting structure of the DNA packaging substrates are summarized in Table 1.

An accessible form of DNA is the defined unit length chromosome produced by ø29. Attached to each 5' end of the 19-kilobase ø29 dsDNA is a covalently linked terminal protein, gene product 3 (gp3) (Mellado *et al*., 1980). This DNA-terminal protein complex, which is analogous to DNA-terminal protein complex in adenovirus (Rekosh *et al*., 1977), is capable of priming of DNA replication from each end, thus providing a straightforward means of overcoming the loss of information in lagging strand synthesis

(Mellado and Salas, 1983). The result is a mature, unit length chromosome that can act as a ready substrate for DNA packaging (Bjornsti *et al*., 1981). Similar to ø29, unit length DNA is produced during replication of the phage P2 genome. Unlike ø29, however, P2 DNA is replicated via a closed circle mechanism similar to plasmid replication (Bertani and Six, 1988). In P2 the covalently closed, circular DNA (Pruss and Calendar, 1978) is processed to a linear form for packaging (Bowden and Calendar, 1979; Bowden and Modrich, 1985). Since this linear molecule must have the capacity to recircularize upon entry into the host cell, the packaging apparatus generates 19-base 5' overhangs that mediate circularization. DNA replication is not so simple in other phages, however, and the substrate chromosome for DNA packaging rarely appears in such an accessible form.

DNA replication during infection by many well-studied dsDNA phages produces a substrate DNA for packaging that is a composite of individual genome lengths organized into head-tail concatemers. Lambda circularizes its infecting DNA molecule via the 12-base pair sticky ends. Unlike in P2, initial closed-circle replication employing a single origin on the DNA is displaced by a rolling circle mechanism that produces DNA concatemers several genomes in length. Thus, to recapitulate the linear chromosome, DNA packaging resolves single copies of the chromosome with the 5' overhangs from the double-stranded concatemer (see below).

The linear virion DNA of many other dsDNA phage types is, unlike ø29, P2 and λ, longer than the length of the genome. In phage Mu, which integrates its DNA into the host cell genome, the additional DNA is of host origin, the result of excision of a length of DNA greater than the length of the integrated phage genome (Harshey, 1988). In

phages T3 and T7, P22, SPP1 and T4, the linear virion DNA is terminally redundant, with a portion of the DNA sequence at one end of the genome being repeated at the other end of the DNA. This terminal redundancy permits replication without the loss of genetic information in that, although linear replication causes loss of information at the 5' ends, the redundancy allows the entire sequence to be recovered during subsequent replication (Keppel *et al*., 1988). These phages employ a variety of mechanisms to generate long concatemers that depend upon this terminal redundancy of the chromosome, which in turn yield terminally redundant genomes during DNA packaging. In some cases (T7, T3) the sequence that makes up the terminal repeat is the same for all virions in the population. In others (T4, P22) the packaging process yields a population of packaged genomes that are circularly permuted with respect to each other and therefore have different terminally redundant sequences in different particles.

While the exact mechanism of replication to form linear concatemers for phages λ, P22, T3 and T7 varies (Keppel *et al*., 1988), the end result is a packaging substrate consisting of a long molecule comprised of multiple copies of the genome from which a virion's complement of DNA is procured during packaging. An additional complication is faced by phage T4, whose invasive strand replication initiation yields not only long concatemers, but ones containing numerous Holliday junctions that leave them highly branched (Dannenberg and Mosig, 1983). These convolutions must be resolved during packaging to yield the appropriate linear DNA to be translocated into the phage head. Phage Mu, whose DNA is integrated into the host genome, must excise copies of its genome from the host chromosome prior to, or concomitant with, DNA translocation

(Harshey, 1988). In each of these systems a complex series of enzymes and processes effect maturation of the DNA substrate and mediate its encapsidation.

Packaging Enzymes

The task of retrieving the phage DNA and processing it to a packagable form rests with a collection of proteins forming a complex often referred to as the terminase holoenzyme (Catalano, 2003). This term belies the primary function of these enzyme complexes in many phage systems where they perform the task of retrieving the unit length DNA packaging substrate from the long concatemers formed by the myriad DNA replication strategies. This definition under represents the true capacities of this group of proteins since it describes only one of multiple functions during packaging. In addition to cleaving the substrate DNA to terminate packaging and generate a new end, terminase complexes target the DNA to the waiting prohead and mediate ATP hydrolysis to power DNA translocation, possibly acting as the primary transducer of force during translocation (see below). The designation terminase does not apply to phages which package a preformed unit length genome, such as ø29, where the enzyme is more appropriately termed the packaging ATPase.

All known terminase holoenzyme (packaging ATPase) complexes function as a complex of two proteins. The classical terminase combination consists of a large and a small protein, each with specific activities. The small subunit recognizes and binds to specific sequences in the substrate DNA in most phage systems and positions the large terminase subunit to cleave the DNA. Endonuclease activity invariably lies in the large

subunit, as well as the ATPase activity responsible for mediating DNA translocation (Hang *et al.*, 2000, Mitchell *et al*., 2002). For most systems the DNA-bound large subunit interacts with the prohead, and hydrolyzes ATP to power DNA translocation.

Proheads

DNA packaging culminates in the insertion of the mature DNA substrate procured by the terminase holoenzyme into a receptive prohead (Figure 1). The icosahedral head shells of the dsDNA phages share the same basic architecture and maturation pathway (Hendrix, 1985). The major shell protein polymerizes to form the prohead shell by associating with the head-tail connector and scaffold-core components. The dodecameric connector (or portal protein) is embedded at one of the twelve vertices termed the portal vertex (Tao *et al.*, 1998). Nomenclature diverges in different phage systems, with the core-containing structure being termed prohead (λ, ø29), prehead (T4), or procapsid (P22). For the sake of consistency, we refer to this precursor capsid shell as the prohead.

There is a common maturation pathway of the prohead for most dsDNA phages that varies only in detail (Figure 1). Putative prohead structures can be isolated from mutants lacking DNA packaging components, and they may or may not contain scaffolding protein. Prohead-like particles may be defective for packaging because they are unstable or immature (Earnshaw and Casjens, 1980), reflecting a need for synchrony of prohead maturation and packaging. For example, expansion of the capsid and concomitant thinning of its wall that is programmed to occur in DNA packaging may have initiated or occurred prematurely. The role of these structural maturation events has

been probed in detail with respect to their mechanistic and temporal relationship with DNA packaging initiation and DNA translocation (see below). In general, the viable receptacle for DNA packaging is the unexpanded, core-containing prohead, with any proteolytic maturation and shell expansion occurring after DNA packaging initiation.

Occupying a unique vertex of the prohead is a multi-functional structure called the head-tail connector or portal that is essential in prohead assembly and DNA packaging (Valpuesta and Carrascosa, 1994). The distinction between these two terms lies in the role this structure plays at different times in assembly. The term portal refers to its role in facilitating the passage of DNA into and out of the prohead, whereas the term connector refers to its role as the junction between prohead and tail. We favor the term connector, simply due to its preference in the systems with which we are most familiar (ø29, T4). Prior to DNA packaging, the connector plays a role in the initiation of head shell formation by interacting with both the scaffold and head shell proteins. In phage T4, the gp20 connector also interacts with gp40 on the inner surface of the cell membrane to initiate head formation (Yap and Rao, 1996). During packaging, the connector binds the mature DNA-packaging ATPase complex, is the portal for entry of the DNA (possibly playing an active role in translocation), and is involved in the signaling for packaging termination. Following the completion of packaging the connector is the target for tail assembly, and in the mature virion it has a role in release of DNA during infection. That the connector is capable of engaging in each of these processes in a precise order speaks to its remarkable capacity to not only do many things, but to do them at the right time.

DNA PACKAGING PROCESSES

In the infected cell viral DNA is recognized by the packaging proteins in a background of host polynucleotides. In spite of differences in the mechanics of DNA replication in different phages, as well as the persistence or absence of an intact host genome, there may be a single mechanism for phage DNA maturation for packaging that is grounded in DNA end formation. DNA maturation for packaging is defined as targeting of the resolved phage chromosome to the waiting prohead. The dsDNA phages select genomic DNA efficiently from the myriad pool of nucleic acids within the cell as evidenced by the high efficiency of infection by progeny, nearing 100% for most dsDNA phages.

Does the DNA-packaging enzyme complex pre-assemble and then target the mature prohead, or does it assemble on the prohead? Is this prohead targeting event correlated with prohead maturation events or with DNA replication or transcription? These points may be crucial in that individual events can be temporally or, more importantly, mechanistically coupled to one another.

Once the prohead and DNA are linked and the DNA is positioned for packaging, how is DNA translocated? The structure and mechanism of the motor and the nature of the chemomechanical energy conversion are the areas of greatest current interest and experimental focus. After the complement of DNA has entered the prohead, packaging is terminated. The unit length of the DNA packaged can be measured by targeting a DNA sequence to signal that the head contains one genome, or the amount of DNA in the head may feedback on the packaging machine to trigger termination. By compiling what is

known for each phage, our intent is to describe a general DNA packaging mechanism for all dsDNA phages. However, a universal mechanism for DNA translocation might not exist, and caution is needed in comparing individual facts from disparate systems.

DNA maturation

Maturation of the phage DNA from the cytoplasm of the infected cell is the first event of packaging (Figure 2). As mentioned, phages such as ø29 and P2 replicate unit length chromosomes. Phages λ, P22, SPP1, T3 and T7, and T4 produce long concatemers of DNA comprised of a number of copies of the genome linked head to tail; unit length chromosomes are cut from these concatemers and packaged.

DNA maturation events in phage λ are quite well understood (Catalano *et al.*, 1995). End formation occurs at the structurally complex *cos* site which spans 200 base pairs of DNA at the ends of the genome. The terminase holoenzyme complex of the two λ packaging proteins, gpNu1 and gpA, binds *cos* through the interaction of Nu1 with three sequence domains, R1 through R3, of *cosB* ["binding"] on the right of the *cos* site. The larger subunit, gpA, then catalyses a single-stranded nicking reaction in the central *cosN* region ["nicking"], in the center of the *cos* site, producing 12-basepair 5' overhangs. GpA is thought to bind as a dimer, thus permitting cutting of both strands on one side of the DNA helix to generate the 12-basepair overhang. Although gpA alone can bind and cut λ DNA *in vitro*, apparently Nu1 is crucial for efficient targeting *in vivo*. Terminase binding and cleavage initiation in λ also involves the action of IHF (integration host factor), which binds the region in the *cos* site between R1 and R2. IHF bends the DNA in

such a way that a dimer of Nu1can bind the juxtaposed R1 and R2 sites (de Beer *et al.*, 2002). Once DNA is cleaved by terminase, strand separation occurs via an ATP-dependent process (Hang *et al*., 2000), possibly to rearrange and activate the terminase subunits bound to the DNA for an additional, as yet unidentified maturation process. Terminase preference for the right side of the *cos* site is driven by gpNu1 binding to *cosB* to form the stable intermediate, complex I. The union of complex I with the λ prohead yields the ternary complex II, which then proceeds to translocate the DNA through the connector and into the capsid.

Termination of λ DNA packaging is achieved by sequence recognition of the downstream *cos* site by the engaged packaging apparatus during DNA translocation. The *cosQ* region on the left of the downstream *cos* complex is recognized, and a *cosB* independent cleavage occurs at *cosN* to generate a complementary 5' overhang on the end of the packaged chromosome (Wieczorek and Feiss, 2001). *cos* cleavage is sequence-specific but also involves detection of the amount of DNA that has been packaged, since constructs with less that 78% of the normal complement of DNA between *cos* sites fail to cut normally (Feiss *et al.*, 1977). This feedback mechanism plays a role in other phages (see below). The termination of each packaging cycle from a concatemer regenerates the complex I, which can initiate a new translocation event. On average, each termination-initiation cleavage event is capable of priming between two and three translocation events without the need to generate complex I from the DNA concatemer *de novo* (Feiss *et al*., 1985).

The other phages requiring a cleavage of their DNA to terminate packaging and to generate a free end for the next cycle display variations of the λ archetype. The

terminase complex recognizes and binds a defined *pac* site on the DNA and catalyses cleavage to generate an end for packaging compatible with requirements for DNA replication on infection. The circular unit-length chromosome of phage P2 is cleaved by terminase at a *pac* site to generate the linear DNA to be packaged (Bowden and Calendar 1979; Bowden and Modrich, 1985). Like λ, P2 terminase endonuclease generates 5' single-stranded ends, 19 bases long. P2 DNA cleavage is coupled to prohead docking in that viable proheads must be present *in vitro* in order for the terminase complex to target and cut the DNA (Pruss and Calendar 1978; Bowden and Modrich 1985). Phages such as T3 and T7, P22, SPP1 and Mu employ a strategy similar to that of λ in that a holoenzyme complex of the two terminase proteins targets a *pac* site. Differences are found in the precision, *i.e.*, the location, of cuts made in the precursor DNA relative to the *pac* site. As in λ, DNA processing accommodates the requirements for DNA replication upon infection. Phages T3 and T7 are proposed to make staggered, defined single-stranded cuts at the *pac* site, but these nicks are 230 and 160 base pairs apart, respectively. It was originally proposed that DNA synthesis separates the strands between these nicks and regenerates double-stranded DNA with blunt ends from the single-stranded ends, ensuring the terminal redundancy needed for genetic competence of the progeny virion (Watson, 1972). More recently, a double-strand break mechanism has been proposed (Fujisawa and Morita, 1997). The terminal redundancy is preserved at the right end via a nicking and replication mechanism in which a displaced template is produced, followed by a double-stranded cut that retrieves the right end of the packaging genome from the concatemer. Packaging terminates with a double-stranded cut at the left end. An

analogue of involvement of IHF in λ packaging appears to be bending of SPP1 DNA mediated by the gp1 small terminase subunit (Chai *et al.*, 1995).

Phages P22 and Mu maintain a *pac* site that is targeted by their respective terminases, but cleave the DNA non-specifically (Mu), or semi-specifically (P22) in a region of the adjoining DNA. In the case of Mu, whose unit-length genome is integrated into the host cell chromosome, the initiation cut is upstream of the phage *pac* site (George and Bukhari, 1981). Therefore Mu retrieves a small portion of the host chromosome DNA, on the order of 56 to 144 base pairs, on the left end of the phage DNA to be packaged. P22 makes an initiation cleavage within a target area of approximately 120 base pairs of its *pac* site, generating a blunt end DNA capable of packaging initiation (Backhaus, 1985; Casjens *et al*., 1987).

In addition to the relative lack of fidelity in initiation cleavage, Mu and P22 do not terminate packaging at a predetermined sequence as in λ or T3 and T7. Rather, these two phages engage in a headful packaging mechanism in which the sequence-independent cleavage of the DNA is determined by the amount of DNA packaged. Packaging of more than one genome length of DNA assures replication competence upon infection. In Mu, host sequences to the right of the phage genome are packaged (Chow and Bukhari, 1978). In P22, 104% of the genome is packaged, providing the terminal redundancy needed for DNA replication (Casjens and Hayden, 1988). How termination cleavage is triggered is unknown, but the physical force of the compacted DNA against some component of the packaging machine, either the connector or the ATPases, may signal that the head is full. Work on P22 and SPP1, that use headful packaging, has demonstrated that mutations in the connector affect the length of DNA packaged (Casjens

et al., 1992; Tavares *et al*., 1992). These mutants suggest that head-full packaging control lies in the connector but leave open the possibility that the terminase complex engaged in packaging could be altered indirectly by these mutations. In SPP1 and P22, as in λ, the termination of the initial DNA packaging event regenerates the initiation complex that can target the next available prohead. Unlike λ, however, the left end of the DNA generated from subsequent rounds of packaging are staggered downstream in increments of 4% in P22 or 5.6% in SPP1 of the genome length as a result of the headful mechanism described above.

Phage T4 is unique in comparison with other well-studied dsDNA phages in that it does not have a defined, sequence-specific *pac* site. The holoenzyme complex of the large T4 terminase protein, gp17, and the small terminase protein, gp16, binds the hydroxymethylated T4 DNA via gp16 targeting. While no particular sequence is recognized, initiation cleavage was recently shown to be coupled to recognition of single-stranded regions generated by replication initiation and transcription (Franklin *et al*., 1998). Gp17 has a domain for binding to single-stranded DNA, and binding is augmented by the smaller gp16 terminase subunit. Therefore, while not sequence-specific *per se*, this requirement for single-stranded DNA suggests that initiation cleavage in T4 is not entirely a random event since it is coupled to sequence specific processes. The large terminase subunit, gp17, interacts directly with the connector, gp20 (Lin *et al*., 1999), and probably the ATPase activity of gp17 powers both DNA cutting and translocation. Recently it has been shown that T4 gp17 also interacts with the phage late sigma factor gp55 (Malys *et al*., 2002), implying that the gp17 terminase subunit

targeting of the DNA is in part directed by a cofactor similar to those seen in λ and SPP1 (see above).

As mentioned, T4 DNA packaging also must resolve a large number of branches in the substrate DNA in order to produce an intact linear DNA genome. Endonuclease VII, gp49, is responsible for much of this work, although gp17 alone might be capable of resolving most branches since some filled heads are produced in the absence of gp49 (Luftig *et al*., 1971). Gp49 works as a resolvase capable of trimming the branched DNA at the Holliday junctions left over from invasive strand-initiated replication (Mizuuchi *et al.*, 1982). Originally it was thought that gp49 acted downstream of the packaging complex on the prohead, but more recently it has been shown that this enzyme is in contact with the gp20 connector during packaging (Golz and Kemper, 1999). Gp49 binds a discrete domain of the connector and is not in contact with the gp17.

T4 DNA packaging is terminated by a headful packaging mechanism, with cutting being mediated by gp17. Isometric, petite heads package DNA to the same density as larger prolate heads, yielding a virion chromosome 40% smaller than normal (Eiserling *et al*., 1970). In addition, canavanine- or head shell mutation-induced lollipop monster phages are capable of packaging large DNA molecules in the megabase range (Cummings *et al*., 1973).

The unit length ø29 DNA with its associated gp3 terminal protein is competent for DNA packaging after replication, without the need for cutting seen in other phages. However, a complex series of steps generates a DNA substrate capable of interacting with the prohead. Sequence independent interaction of the terminal protein, gp3, with the downstream DNA helix has been described (Grimes and Anderson 1997). This produces

lariat loops that can form without the participation of other phage proteins. Only one gp3 is required, since lariats can form from various lengths of either right- or left-end fragments of the DNA generated by restriction endonuclease digestion. Binding of the gp16 packaging ATPase to gp3 at the lariat loop junction permits the introduction of supercoils into the lariat. Whether this supercoiling event is ATP-dependent is unclear, and the mechanism by which the DNA is twisted has not been resolved.

This higher order conformation of the DNA-gp3-gp16 seen in ø29 likely provides efficient packaging initiation. It has been demonstrated that free ø29 connectors wrap about 1.6 turns of DNA around their outside surface (Turnquist *et al*., 1992), and gel electrophoresis and electron microscopy show that the connector embedded in the intact prohead also binds and wraps supercoiled DNA (D. Anderson, unpublished). This event provides a mechanism by which the end of the DNA is targeted to the connector portal to initiate packaging. Considering that the end of the mature chromosome is a relatively small target in the busy environment of the cell, the long axis of the DNA is a large target for any complex capable of recognizing and interacting with the DNA. We suggest that ø29 takes advantage of this by targeting the long axis of the DNA and then moving in one dimension to the end to initiate translocation. Whether nicked DNA, being torsionally unconstrained, can initiate packaging has not been clearly resolved. It is also unknown whether such DNA tertiary structure is employed by other dsDNA phage as a means of targeting the DNA-packaging enzyme complex to the prohead.

A second possible function for the wrapping of supercoiled DNA around the connector is to effect connector conformational change that converts it from a static organizer of shell assembly to a dynamic packaging motor organelle. This idea is based

on the finding that wrapping of supercoiled plasmid DNA around the ø29 prohead-embedded connector allows the shell, previously tightly fixed to the connector, to be easily stripped away. The contour length of the DNA-connector complex that remains is reduced by about 120 base pairs, suggesting that DNA wraps around the connector and restrains a negative supercoil, as demonstrated previously for the free connector-supercoiled DNA complex (C. Peterson and D. Anderson, unpublished; Turnquist *et al.*, 1992).

Prohead Maturation

A common theme in dsDNA phage head assembly is the maturation of the prohead from a fragile, scaffold/core-containing precursor to a stable mature form. The head shell likely polymerizes around a scaffold-connector complex in a relatively unstable form, which later transforms to a rigid and structurally robust capsid (Earnshaw and Casjens, 1980). This structural conversion can involve proteolytic cleavage of the scaffold and/or shell proteins and an increase in the prohead volume by as much as 100% (HK97; Conway *et al.*, 1995), a process called expansion. Scaffold exit may precede or occur concurrently with expansion.

While both scaffold exit and capsid expansion (with the exception of ø29, which does not expand) must occur to allow the full complement of DNA to enter the prohead, the question of whether they contribute directly to DNA translocation persists (see below). Considering scaffold processing, early models of DNA translocation focused on the compacted phage chromosome as an analogue of the condensed DNA produced by

polyvalent cation-mediated collapse of DNA into a toroid (Laemmli, 1970). In some phages, such as T4, it was suggested that core cleavage might produce small, charged peptides capable of condensing the phage chromosome within the head, thus drawing the long linear DNA through the portal vertex (Laemmli, 1970). Studies on phages T7 and P22 suggest that the core exit may be coupled to DNA packaging, since only core-containing particles are packaged *in vitro* (King and Casjens, 1974; Roeder and Sadowski, 1977). *In vivo* observation of T4 offered additional support in that only core-containing particles could be chased into phage during both wild-type and mutant infection (Laemmli and Favre, 1973; Bijlenga *et al.*, 1973; Luftig and Lundh, 1973). On the contrary, λ proheads can package DNA after core exit (Hendrix and Casjens, 1975), and ø29 proheads containing only about five copies of the normal complement of about 150 copies of scaffolding protein are packaged efficiently *in vitro* (D. Anderson, unpublished). These conflicting observations have not been reconciled, and there is no current mechanistic model relating scaffold exit and DNA packaging.

Is the structural transformation of the lattice in prohead expansion mechanistically linked to DNA translocation? Only unexpanded proheads of λ, T7 and P22 can package DNA *in vitro*, and expansion occurs during packaging (Earnshaw and Casjens, 1980; Shibata *et al.*, 1987). This led to the suggestion that prohead expansion might drive DNA translocation. Decrease in head shell ion permeability during expansion in T7 prompted the hypothesis that the DNA might be sucked into the sealed prohead by the hydrostatic pressure created during expansion (Serwer, 1975).

However, though increase in head shell volume is dramatic, for example, on the order of 50% in T4, this increase is insufficient to account for the amount of DNA

translocated into proheads exhibiting capsid expansion (Earnshaw and Casjens, 1980). DNA translocation is ATP-dependent for all phages, and no link between prohead expansion and ATP hydrolysis has been described other than the synchrony of packaging and expansion. In addition, ø29, which exhibits capsomere structural change in packaging, does not show a detectable increase in shell volume (Tao *et al*., 1998). In phage T3, which initiates packaging into unexpanded proheads, expansion occurs discretely after a small portion of the DNA complement is translocated (Shibata *et al*., 1987). Similarly, the capsomere change in ø29 probably occurs after only a few hundred base pairs of the DNA enter the prohead (Bjornsti *et al*., 1983). In T3, packaging can be stopped *in vitro* after capsid expansion with the addition of ATP analogues such as gamma-S-ATP, and then restarted and completed into the expanded prohead upon restoration of ATP (Shibata *et al.*, 1987). The hydrostatic pump model has been revised such that a regenerated hydrostatic pressure is maintained across the prohead shell which pulls the DNA into the capsid (Serwer, 1988). This model still suffers, however, from the observation that report effective translocation can occur after initial prohead expansion (Shibata *et al.*, 1987; Rao and Black, 1985) and that expansion is most likely irreversible (Steven *et al.*, 1992).

The singular test case that unlinks expansion and DNA packaging is the report of *in vitro* packaging of a phage T4 particle after both core cleavage and expansion (Rao and Black, 1985). However, T4 DNA packaging *in vivo* cannot occur after scaffold cleavage and shell expansion (Luftig and Ganz, 1972). Temperature shift experiments with temperature-sensitive and cold-sensitive mutants in the T4 terminases show that the expanded prohead is not rescued *in vivo.* The only proheads that have not initiated

packaging that can be rescued in similar experiments are scaffold-containing, unexpanded proheads (Kellenberger 1980), implying that after scaffold cleavage, the prohead proceeds down a defective pathway *in vivo*. To credit the *in vitro* experiments requires the assumption that expanded proheads be rescued only with transfer into the *in vitro* world. Additionally, expanded T4 proheads assemble tails *in vivo* without packaging DNA during infection with terminase mutants (Jardine *et al*., 1998). This suggests strongly that expansion prior to DNA packaging is aberrant. It has been shown recently that the normal *in vivo* substrate for DNA packaging initiation in T4 is the unexpanded prohead, and that prohead expansion occurs after a significant amount of DNA enters the prohead as in other phage (Jardine and Coombs, 1998). The newly reported high efficiency *in vitro* T4 packaging protocol (Malys *et al*., 2002) can be used to retest the activity of the expanded prohead.

If DNA packaging is not mechanistically coupled to head maturation events, what is the impetus for scaffold exit and prohead expansion in DNA translocation? The answer may lie in subtle events of packaging initiation and early DNA translocation that involve connector conformational change, and consequently, irreversible conformational change in the shell. Ordered virion assembly is assured by binding the DNA packaging apparatus to an unfilled prohead, but not a filled particle. Thus, DNA packaging is coupled temporally to core cleavage and prohead expansion so that packaging precedes tail attachment.

How DNA packaging triggers prohead expansion is unknown. Expansion of the T4 polyhead lattice *in vitro* under conditions of low salt is unidirectional and exothermic (Steven *et al.,* 1976, 1992). It is hypothesized that enough DNA to form a single layer

within the prohead, contacting the inside of the head shell, might trigger expansion. However, apparently much less DNA packaged, on the order of a few hundred base pairs, is thought to trigger capsomere conformational change in ø29, albeit without expansion. Expansion might also play a role in ensuring proper organization of the packaged DNA by opening new binding sites on the inner surface of the capsomeres. Possibly changes in the connector may propagate a wave of expansion up the head. Connector conformational changes likely mediate packaging initiation, establish the sensing mechanism for headful packaging in some phages, and key ordered tail attachment. A certain consequence of shell expansion is to provide stability to both the nascent and mature virion.

The Mechanism of DNA Translocation

Union of the mature substrate DNA and the ATPase (terminase) holoenzyme complex with the viable prohead results in the assembly of a molecular machine that is capable of translocating DNA into the prohead. After decades of effort, the exact mechanism of DNA translocation is unknown. Key in the search for the mechanism of DNA translocation is that ATP hydrolysis is the driving force behind DNA translocation in all *in vitro* systems. Moreover, all identified DNA packaging holoenzyme complexes have the capacity to hydrolyze ATP. Therefore, the task is to define where, when and how ATP hydrolysis is used to move DNA around or through the connector and into the head.

First and most poorly understood is the mechanism by which the free end of the double-stranded DNA substrate, once engaged with the head-tail connector, is introduced into the portal pore. While DNA deposition may involve a continuous transfer from the engaged ATPase complex through the connector pore, this delicate initiation must depend on the highly evolved fidelity of the terminase holoenzyme and connector.

The mechanism of DNA translocation relates broadly to how molecular machines in general harness biochemical events to achieve movement of molecules. Included in the list of well-studied motors are the myosin ratchet, the F1-ATPase rotary motor, and the RNA and DNA polymerases. The same questions that persist in these motors apply to the DNA translocation motor: Is there a bias to trap favorable Brownian motions by a sequence of small free energy drops [power stroke], or is a run of favorable thermal fluctuations rectified by a large free energy drop [Brownian ratchet] (Oster and Wang, *in press*).

At the center of past efforts in describing the mechanism of DNA translocation lie the ATPases themselves. The simplest, and most appealing, mechanism is one in which the terminase holoenzyme plays a direct role in translocation. It has been suggested that the oligomeric ATPase holoenzyme associated with the connector during DNA packaging might be able to oscillate up and down with respect to the axis of the DNA entering the connector (Fujisawa and Morita 1997). In a mechanism similar to the myosin head ratchet, the ATPase subunits would walk, either individually or in concert, unidirectionally along the DNA helix, and the DNA would be translocated in the process (Figure 3). The precise nature of the structural transitions in the ATPase complex that are required to fulfill this mechanism have yet to be described.

Similarly, the ATPase holoenzyme complex could directly translocate the DNA into the prohead via a mechanism similar to polymerase tracking along the DNA. If the ATPase complex is fixed at the connector portal and creeps along the DNA like a polymerase, the result would be translocation. This polymerase-creeping model and the similar ATPase ratchet model draw support from the inhibition of packaging by DNA intercalating compounds and the detected ability to package DNA with gaps or nicks. This implies that the exterior phosphate backbone of the DNA is the point of interaction between the translocating motor and the substrate (Fujisawa and Morita, 1997). Whether the ATPase holoenzyme plays a direct role in energy transduction or not, the hydrolysis of ATP catalyzed by the terminase complex plays a defining role, as evidenced by the effect of certain mutations in the ATPase region of the λ gpA protein on the rate and efficiency of translocation *in vivo* (Duffy *et al*., 2002).

Many current models of DNA packaging hypothesize a role for the symmetry mismatch between the dodecameric connector and the five-fold symmetric vertex of the icosahedral shell in which the connector is embedded (Figure 4a). This symmetry mismatch potentiates rotation of the connector within the prohead shell (Hendrix, 1978) by abrogating the rigid interaction of components of like symmetry. The symmetry mismatch of the connector and capsid is confirmed by cryo-electron microscopy 3D reconstruction of ø29, which also shows that the connector appears to fit loosely in the shell (Tao *et al.*, 1998). Models have been put forward in which connector rotation either actively drives packaging or passively facilitates packaging.

The original connector rotation model of packaging has the helical DNA being driven into the capsid, with either active or passive rotation of the connector, as a bolt

passes through a rotating nut (Figure 4b, Hendrix 1978). It is not clear how an active screw model would satisfy certain requirements of this mechanism, such as the need to axially restrain the DNA to prevent it from being rotated by the connector. Later, the observation that the ø29 connector could wrap supercoiled DNA prompted a model in which a rotating connector would move the externally wrapped DNA relative to the head shell (Turnquist *et al.*, 1992). This physical displacement could be harnessed in a number of ways to produce DNA translocation, including direct translocation into the prohead similar to a ship's capstan. Alternatively, twisting of the DNA causing the introduction of supercoils into the DNA by the rotating connector or by a packaging ATPase activity (Black and Silverman 1978) could put strain on the DNA so that it enters the prohead to eliminate this superhelical stress. As yet there is no idea of how movement between the connector and shell are mediated in the active connector rotation models, and connector rotation has not been detected in any phage system. The recent atomic structure of the ø29 connector has generated a model of the packaging mechanism that combines the ratchet and rotation models (see below).

A model that introduces a caveat to the active ATPase models above is one in which the head-tail connector of the phage might move along the DNA backbone in order to achieve DNA translocation. In rationalizing the observed 13-fold symmetry of the free SPP1 connector, Dube *et al.* (1993) proposed a mechanism of translocation in which monomers in the 13-fold portal interact in set sequence with the near 10-fold symmetric DNA helix. As monomers in set sequence bind the backbone of the helix, the DNA must be drawn into the prohead as the perpendicular alignment of the connector and DNA is maintained (Figure 5). This model was based on a 13-fold model for the SPP1 connector,

which has since been shown to be a dodecamer in the prohead like other dsDNA phage connectors (Lurz *et al.*, 2001). However, the twelve-fold nature of the connector does not exclude this model.

The ø29 connector structure reveals a most interesting motif. Each of the twelve monomers spans the 75Å high connector from top to bottom (Simpson *et al.*, 2000; Guasch *et al.*, 2002). However, rather than simply traversing the connector, each monomer has three nearly parallel alpha helices that are canted at an angle approaching 30° to the axis of the connector, giving the overall structure the appearance of a spring. The structure of the connector therefore gives the impression that it is compressible. While no comparative structures have been presented to show connectors of different heights, atomic force microscopy has revealed that the connector can be reversibly compressed by 25Å, about one third of its height, under loads of 100 picoNewtons or more (Muller *et al.*, 1997). The connector structure and this demonstrated compressibility serve as the basis for the translocation model described below.

It is proposed that the connector oscillates, extending and contracting along the long axis of the DNA inserted in the connector channel (Figure 6; Simpson *et al.*, 2000). There are two primary contact regions between the connector and DNA, at the connector wide and narrow ends, respectively. To start a cycle, the DNA is released by the connector narrow end which rotates by 12° (counterclockwise as viewed toward the head) to maintain contact with the DNA backbone as it extends down the DNA helix by two base pairs. Then the narrow end closes on the DNA as the connector contracts to drive the DNA into the head, and concurrently the connector wide end releases the DNA and rotates 12° to realign the connector with the prohead, pRNA and gp16 ATPase. These

components are reported to be in contact and possess five-fold symmetry as suggested by cryo-EM. This cycle repeats. The connector rotation involved is passive and serves to maintain alignment between the six-fold connector and five-fold DNA. How ATP hydrolysis mediates these events is unknown, and no direct quantification of the connector dynamics involved has been reported.

STRUCTURE OF PACKAGED DNA

Regardless of the mechanism of DNA translocation, it must overcome the energetic barrier of compacting the DNA and deliver the DNA into the prohead to confer the proper structure and organization within the head. DNA packaging in dsDNA phages is endothermic. Analogies are often made between DNA packaging and the collapse of DNA into a torus, or DNA toroid, in the presence of polyamines such as spermidine (Eickbush and Moudrianakis, 1978) or hexamine cobalt (Widom and Baldwin, 1980). While the final structures share structural similarities, such as the organization of DNA in hexagonal bundles, the processes are very different. DNA condensation by polyamines or $Co^{3+}(NH_3)_6$ is spontaneous and exothermic, while DNA compaction in phages requires the input of energy mediated by enzymatic function of the packaging machine. The DNA toroid is stable, while the packaged DNA in phages is metastable. This distinction is crucial, since the function of the phage packaged DNA is to await delivery into a host cell, and it must be ordered in such a way as to permit disassembly of the structure during infection, unlike the DNA toroid which may be irreversibly condensed. Therefore, DNA packaging is defined as a compaction event rather than a condensation.

Several facts seem incontrovertible in describing the structure and organization of the DNA in the phage head. The DNA is in B-form (Aubrey *et al*., 1992), with spacing on the order of 25Å (Stoud *et al.*, 1981). Regardless of the specifics of the overall organization of the DNA, it would appear that it is locally associated in a hexagonal packing array, giving the DNA a quasi-crystalline appearance. What has been debated significantly is the overall organization of the DNA within the head shell. Models proposed over the past four decades describe the gross features of packaged DNA as a wrap solenoid, a liquid crystal, a spiral fold or a folded toroid (Figure 7). Until recently, insufficient, and often conflicting, experimental data existed to distinguish among these models. However, mounting evidence pushes consensus in the direction of one of the original models of DNA organization in the phage head, the DNA solenoid.

The model for packaged DNA structure that seemingly has the least amount of organization is the liquid crystal model (Lepault *et al.*, 1987). In this model as in others, the bulk of the DNA is in tightly packed crystalline arrays. These arrays are small, however, and persist within the phage head as discrete domains with local structure, joined to other randomly arranged packets of ordered DNA by short stretches of disordered DNA. The DNA becomes organized in this fashion as more and more DNA is translocated into the prohead, and the DNA condenses in small regions into hexagonally packed crystals. This is an intuitively appealing model, but much of the experimental observation made regarding DNA structure within the phage head appears to be in conflict with this model.

Microscopy studies suggest there is higher symmetry to packaged DNA beyond the level of hexagonal packing promoted in the liquid crystal model. DNA released from

disrupted heads often appears as a large coil, suggesting a gross organization of the whole chromosome (Earnshaw *et al*., 1978). Cryo-electron micrographs of several phage heads and similar structures reveal patterns in the compacted DNA which resemble fingerprints, suggesting a pattern of loops of DNA inside the head (Cerritelli *et al*., 1997; Schmutz *et al*., 1999). Three models considered below that describe gross organization of the DNA in the head have experimental support. What is crucial, however, is not just a model of how the DNA can be accommodated in the space of the prohead shell, but one that also accounts for the DNA translocation event.

One model for packaged DNA is based on a derivative of the condensed toroid (Hud, 1995; Hud and Downing, 2001). The DNA enters the prohead and forms a large donut-shaped structure of hexagonally packed DNA with a hole in the middle, essentially a DNA toroid. As the length of the DNA inside the prohead increases, the toroid collapses into a folded structure, and the packaged DNA density reaches the level found in the mature head. But this attempt to relate the structure of the toroid with packaged DNA fails in that the diameter of the torus that collapses into the folded form inside the head is much greater than the diameter of the prohead shell itself. To arrive in this final form, the DNA must organize into the folded toroid from the outset, or a smaller torus having the size of the head must rearrange into a larger toroid ring of the diameter of the final folded form. These events are unlikely, especially with the requirements for gross rearrangement and the energetically unfavorable sliding of DNA. Thus, while this model may have merit in describing how DNA can fit into the prohead, it does not address the obligate process of how DNA will reach this form during DNA packaging.

A second and earlier model that was derived from experimental observation is the spiral fold model (Black *et al*., 1985; Black, 1989). Like others, this model describes hexagonally packed DNA, but unlike the liquid crystal model, the entire chromosome is organized. Briefly, the DNA is arranged as a bundle of straight rods formed by the up and down winding of the DNA along the long axis of the prohead, with the DNA bending back on itself repeatedly, making 180° turns. Several lines of evidence support this model. A series of cross-linking experiments in lambda in which bis-psoralen agents are used to interrogate the detail of interaction between packaged DNA and the phage head shell suggest that the DNA contacts the head shell every several hundred base pairs (Widom and Baldwin, 1983). Thus the kinked DNA at the end of each spiral fold contacts the shell. Ion etching experiments on λ (Black *et al*., 1985), capable of probing the spatial arrangement of DNA within the virion, also support this model.

The spiral fold model, like the folded toroid, presents a reasonable form for the DNA in the full head but is counter-intuitive with the nature of DNA translocation. The sharp 180° bends in the DNA described by the spiral fold require the DNA helix to melt at these points. Considering that the DNA is processively driven into the head, it is difficult to imagine what would force the first complement of DNA to align in this way with such severe distortion of the helix. Seemingly the DNA would prefer to trace a path around the sphere of the prohead rather than reverse direction and fold back on itself, since doing so would deny the high persistence length, charge repulsion and entropic nature of DNA. Recently, new *in vivo* intra-capsid DNA cleavage experiments (Mullaney and Black, 1998) have led to a slight revision of the spiral fold, bringing it more in line with the solenoid packing model (below).

A third model of packaged DNA, the solenoid, has received ongoing attention for four decades. This model has been reinvigorated by recent support derived from cryo-electron microscopy, which is capable of revealing relatively fine detail without the impediment of artifacts generated by fixation or staining seen in traditional transmission electron microscopy. In most phages, raw images of filled heads or virions reveal a characteristic fingerprint pattern. Cerretelli and others (1997) exploited the ability to preferentially orient phage T7 heads in vitreous samples, revealing that all T7 heads have this characteristic pattern. By processing images of these oriented head particles and by regenerating similar images using a theoretical model of a DNA solenoid, some of the best evidence is provided that DNA in phage heads is organized in a layered spool. Assessment of the DNA secondary structure of T7 and other phages by RAMAN spectroscopy also is seen to support this model (Overman, 1998), as does recent theoretical work (see below).

As mentioned above, the key consideration in exploring the structure of packaged DNA is the nature of DNA itself. First, DNA is stiff and has a relatively long persistence length: DNA in solution does not bend back on itself over a distance of less than fifty nanometers. Second, the highly charged phosphate backbone of DNA makes it self-repulsive: in order to compact DNA to a spacing of 25Å and within the confines of the phage head, this charge repulsion must be overcome and will play a role in determining the organization of packaged DNA. Considering these conditions and the rules of entropic confinement allows for a physical reconstruction of packaged DNA that supports the solenoid model.

Providing more detail, when DNA enters the prohead, its stiffness and self-repulsive nature dictate that it remain relatively straight over the short distance from one side of the prohead to the other. When a length of DNA equivalent to several lengths of the prohead has been translocated, how will the DNA respond? It is likely that the DNA will form loops within the confines of the prohead, following the longest path it can around the inner surface of the prohead shell. As more and more DNA enters the prohead, concentric shells of DNA form, pushed outward from the center of the prohead, driven by the persistence of the DNA. As these layers form, charge repulsion between strands pushes back sequentially from layers at the outside of the shell. Thus the properties of DNA itself are enough to confer some level of higher order structure of the packaged DNA in that a stable equilibrium forms between the persistence of the DNA, which pushes the DNA away from the center of the prohead, and charge repulsion that pushes back. The model of such a solenoid structure formed in this fashion is one of the oldest proposed for packaged phage DNA, but only recently has the model been dealt with theoretically to a suitable degree.

Odijk (1998) has calculated the spacing prescribed by such a model and compared it with the observations made by Ceritelli *et al.,* (1997). It appears that the spacing observed in T7 heads containing different amounts of DNA agrees well with the theoretically derived spacings based on the principle of equilibrium between DNA stiffness and self-repulsion. Kindt *et al.* (2001) took this theory one step further in an attempt to replicate the way in which DNA is organized in the prohead during translocation. Their dynamic model interrogates how DNA organizes itself during translocation and not simply after the entire DNA complement has entered the prohead.

This effort reveals that, at first, the DNA is disordered inside the prohead shell, but soon adopts the conformation of the outer layers of a solenoid. As more and more DNA enters the confines of the head shell, concentric layers form from outside to inside of the solenoid. Although the size of this theoretical prohead deviates from real phage systems by several fold, the principle supports the idea that the physical nature of DNA alone can organize the DNA within the prohead.

End state of the packaged DNA also relates directly to the mechanism of DNA translocation in another way. For example, if the final conformation of the DNA inside the capsid shell is a solenoid, then as the DNA enters the prohead through the connector it must rotate axially with respect to the prohead as the incoming DNA winds in concentric coiled rings. A variant of the solenoid proposed by Serwer (1986) abrogates this necessity in that it was proposed that rather than spooling continuously in one direction, the DNA reverses direction occasionally in its path around the solenoid. If no such reversals occur in the solenoid, then the translocation mechanism must accommodate the axial rotation required. The rotation of the prohead connector potentiated by the symmetry mismatch between prohead shell and connector leaves open the possibility that, regardless of the details of the mechanism of translocation, such required DNA rotation can be accommodated. Such a rotation event could also occur, or might be necessary, during DNA ejection during infection. In a reversal of packaging translocation, the connector and attached tail components might rotate relative to the head while the DNA moves into the host cell.

The final structure of the DNA brings up an additional point: how much energy is invested in the DNA? This is relevant to the energetics of DNA translocation in that the

packaging machine must maintain the capacity to drive the DNA into the prohead. Recent single molecule optical trap studies with the ø29 system reveal the force-velocity relationship of the packaging motor (Smith *et al.*, 2001). The DNA packaging machine is remarkably strong at the molecular level, having a maximum stall force on the order of 75 picoNewtons. By comparison, this is five times stronger than the classical myosin molecular motor (Kinosita *et al*., 1998). Why is the packaging machine so powerful? Force-velocity measurements reveal that the last portion of the ø29 chromosome enters the prohead against an internal force of 65 picoNewtons. This suggests that the pressure of the packaged DNA within the prohead, and thus the force opposed in translocating the last segment of DNA, is on the order of 6 megaPascals. Previous studies suggest that the containment pressure of the packaged DNA might assist in ejection of the DNA into the infected host cell. The ø29 work suggests that a significant amount of energy is available for such a process. However, the ø29 single molecule studies do not discern whether all of the energy consumed by the packaging machine is deposited in the packaged DNA, or whether some energy is dissipated. Theoretical estimates for the stored energy of the packaged DNA vary, and it is not clear whether the experimental set-up of the optical trap affects the packaging motor. However, the internal capsid force estimated in ø29 agrees quite well with force calculated for expulsion of T7 wild-type versus deletion mutant DNAs in titration calorimetry (Raman *et al.*, 1997).

CONCLUSIONS AND FUTURE CONSIDERATIONS

The efforts and information described above are directed toward a single goal: the complete understanding of the processes and events of DNA packaging in dsDNA phages. While much progress has been made, many questions remain. The elucidation of the mechanisms involved in DNA packaging can be tackled like any other molecular mechanism by following the "path to enlightenment" (Alberts and Miake-Lye, 1992) which requires the following: 1) a complete list of components, 2) the description of all intermediates, 3) the kinetics of all reactions, and 4) atomic structures of all components. Some of the gaps in this effort include those listed below.

Have all the components in the DNA packaging reaction been accounted for? Recent description of the role of the T4 late sigma factor, gp55, in DNA packaging (Malys *et al.*, 2002) suggest that we should be ever vigilant for missing components which may play a vital mechanistic role in DNA packaging. To date, only a single instance of the involvement of a packaging RNA has been described in ø29. The onus is on investigators in the field in general to seek out and remain open to similar new components in all systems under study.

An ongoing controversy deals with the interaction of individual processes, such as prohead maturation and the initiation of DNA translocation, and whether such events are mechanistically coupled. To completely understand any given individual event, we must be aware that processes might depend upon, and in fact be driven by, other seemingly independent processes.

Many of the unknown factors that remain to be investigated relate to the structure and interaction of components. Among these is the question of the order of assembly of packaging ATPase components, DNA substrate and receptacle prohead. Is the order of

assembly *in vivo* described in λ the same in other systems, with the complex I structure, comprised of terminase and DNA, forming separately from the prohead? Does the prohead play an integral role in DNA maturation, as appears to be the case in P1? Can such interactions help describe the control of DNA processing from concatemers within the crowded environment of the host cell and the mechanism of DNA maturation prior to translocation?

A considerable effort has been directed toward solving the structures of components of the DNA packaging machine at high resolution. Much work remains to be done. The singular example of the solution of the ø29 connector structure must (and will) be matched with connectors from other systems. Solution of the structure of packaging ATPase subunits is crucial in building a complete mechanism of their action (de Beer *et al*., 2002). Atomic resolution of the prohead with embedded portal is as important. Finally, a complete picture of packaging will only be available when structures of all the various intermediates are solved, including the solution of an intact DNA packaging machine at various points in the ATP hydrolysis cycle.

Broader issues precede such atomic level concerns. What is the role of symmetry between components in the DNA packaging motor? Does rotation of components play a role as described in several models described above? Will new motifs for molecular motors be revealed as we approach complete elucidation of the mechanism of DNA packaging?

As a relatively mature discipline within molecular biology, research on phage DNA packaging has enormous advantages. The genetics for most systems are well established and the production of large quantities of materials is relatively easy,

particularly when compared to eukaryotic systems. This puts the study of dsDNA packaging in the enviable position of being primed for the application of new technologies within the fields of biology and biophysics. Among these are single molecule approaches based upon optical tweezers and atomic force microscopy. Advanced spectroscopy, including fluorescence, RAMAN, EPR, FRET and many others, seem tailor made for many of the questions waiting to be answered about the processes involved in packaging. As well, advances in soft matter physics seem newly capable of providing theoretical insight into the problems and mechanisms involved in DNA packaging, perhaps allowing a return of phage research to its roots in physics.

Lastly, the context with which current and future results are taken must continually be brought back to their cellular origin. As complex as these processes might seem *in vitro*, they are perhaps more complex in the *in vivo* world. This context is crucial if we expect to fully understand these events and processes, and later apply them to other areas of interest.

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Figure Legends

Figure 1. Schematic of generalized dsDNA phage assembly. A prohead interacts with the packaging ATPase holoenzyme-DNA complex via its head-tail connector. ATP hydrolysis powers translocation of the mature DNA, and at some point the scaffold core is ejected, either whole or following proteolysis. After an amount of DNA enters the head the shell capsomeres rearrange, making the head more angular and, in most phages, increasing the head volume. DNA translocation continues until a full complement of DNA enters the head, determined either by the unit length of the DNA, sequence recognition of the DNA length, or a headful mechanism. The ATPase-DNA complex detaches from the connector and is replaced by neck, tail, and/or tail fiber components, yielding a mature, infectious virion.

Figure 2. Schematic of selected strategies for DNA maturation and packaging. Maturation ranges from formation of defined unit length chromosomes in λ, ø29 and P1, to terminally redundant SPP1 and T4. Mu retrieves DNA from the host genome upon excision. Structure of the *pac* site and accuracy/location of cleavage (arrows) with respect to *pac* varies among phages.

Figure 3. An ATPase ratchet model of DNA translocation in T3. A gp19 ATPase subunit positioned on the connector interacts with the phosphate backbone of the DNA (a). Upon hydrolysis of ATP, the gp19 monomer changes conformation (b), driving the DNA through the connector and into the prohead. This translocation event brings the

backbone into alignment with the next consecutive ATPase monomer (c) to initiate the next translocation event (d). Reproduced from Fujisawa and Morita, 1997, with permission.

Figure 4. Symmetry mismatch between the prohead and connector potentiates connector rotation and DNA translocation. Due to the 5-6 mismatch, only a single vertex of each component is aligned at any one time (a), thus facilitating rotation. If the contact point becomes a lever for translation of the connector with respect to the prohead (*), rotation can be driven by successive events around the subunits. If the connector acts like a nut and the DNA helix like a threaded bolt, connector rotation could displace the DNA into the prohead (b). Reproduced from Hendrix, 1978, with permission.

Figure 5. Translocation of DNA based on connector rotation and symmetry mismatch between the connector and the DNA in SPP1. Contact (arrow) between a monomer of the 13-fold connector and the phosphate backbone of the DNA helix (a) is broken and the connector rotates 11° counterclockwise. Contact between the connector and the helical DNA is reestablished three connector monomers to the right, two base-pairs down the helix (b), with a consequential translocation of these two base-pairs. Reproduced from Dube *et al.*, 1993, with permission.

Figure 6. Schematic of the compression ratchet model of the packaging mechanism. A packaging stroke begins (a) with the alignment of the wide end of the connector with the prohead (gray circle) and the connector channel with the DNA helix (gray triangle); the

connector is in a compressed form (right). As the connector narrow end releases the DNA (the wide end holds the DNA) and extends (b), it rotates by 12° counterclockwise with respect to the head such that contact with the DNA shifts to the next pair of connector monomers (left) and two base pairs down the DNA helix (right). During the subsequent compression of the connector (c), the DNA, axially restrained at the narrow end (but released at the wide end), is driven two base pairs into the head (right); concurrently, the wide end of the connector rotates passively 12° counterclockwise with respect to the head (left), reestablishing contact with the head two connector monomers to the left. Reproduced from Grimes *et al.*, 2002, with permission.

Figure 7. Schematic of sectional views of models of DNA packing in dsDNA phage virions. All proposed models of DNA packing include hexagonally packed DNA, and differ according to the extent and type of global organization within the head shell. Models for global organization include (a) the solenoid, (b) the spiral fold, (c) the liquid crystal, and (d) the folded toroid.

Table 1. Viral DNA and DNA Replication Strategies of Various dsDNA Phages

Phage	Incoming Virion DNA	Replication Strategy	End State of Replicated DNA
λ	linear with 5' 12-base complementary ends (cohesive ends)	closed circle switching to rolling circle	linear concatamer
P22	linear with 104% terminal redundancy	recombination with extension via direct repeats	linear concatamer
ø29	linear with covalently attached gp3 at 5' ends	gp3-primed extension, strand displacement	unit length with gp3 covalently attached at 5' ends
T3 (T7)	linear with 230 (160) base pair direct repeats	recombination with extension via direct repeats	linear concatamer
T4	linear with 102% terminal redundancy	invasive strand initiated via terminal redundancy	branched concatamer
SPP1	linear with 104% terminal redundancy	unknown	linear concatamer

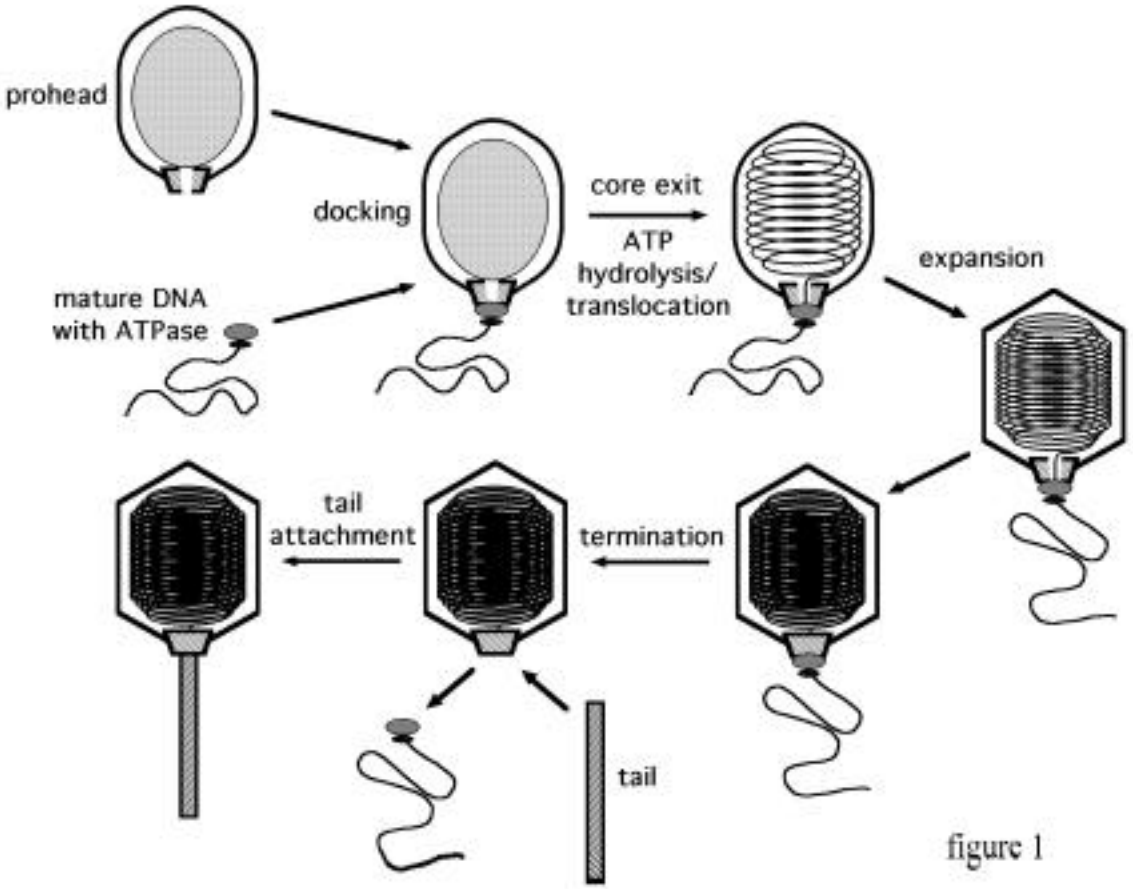
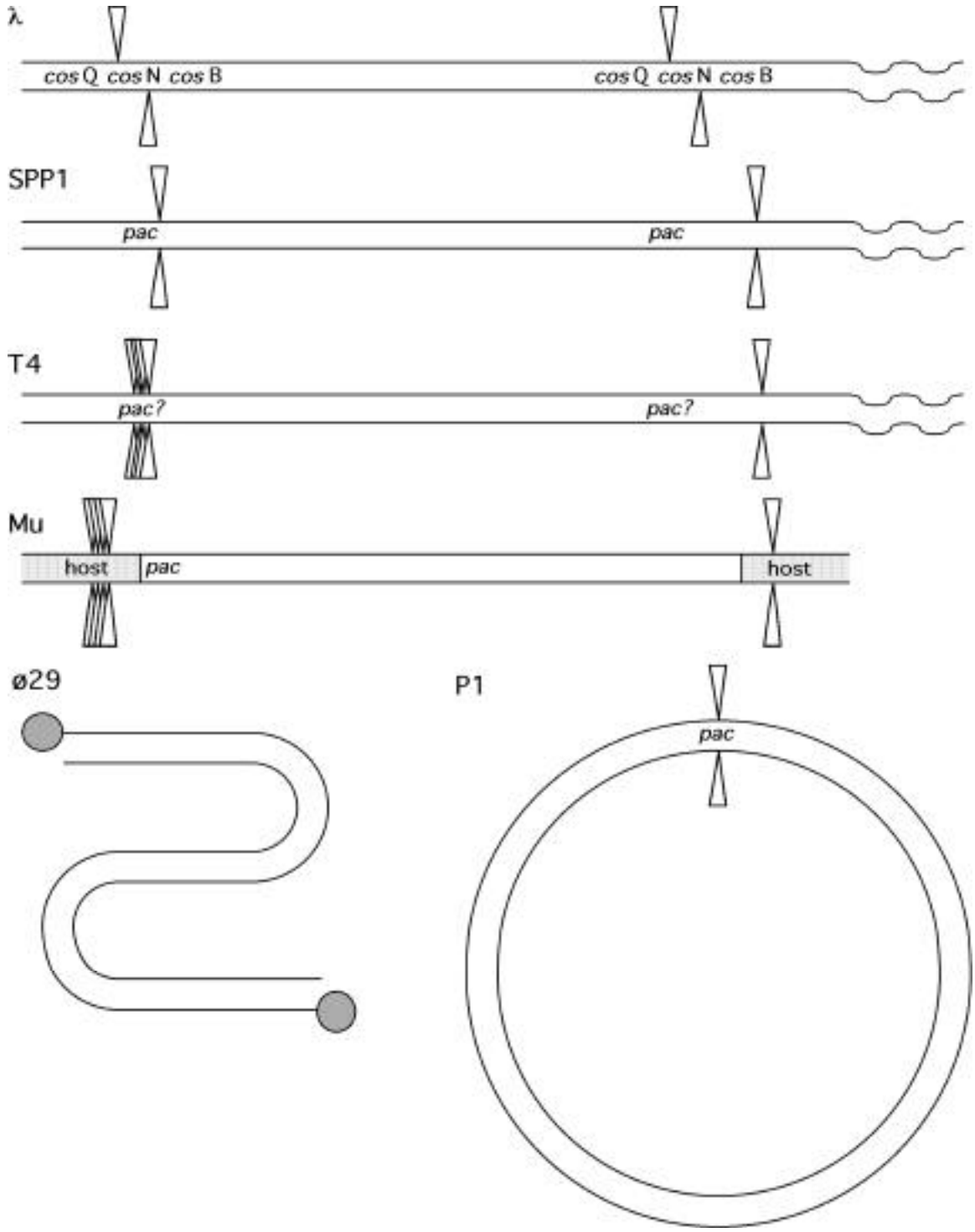

prohead
docking
core exit
ATP
hydrolysis/
translocation
expansion
mature DNA
with ATPase
tail
attachment
termination
tail

figure 1


λ
cos Q cos N cos B
cos Q cos N cos B
SPP1
pac
pac
T4
pac?
pac?
Mu
host
pac
host
ø29
P1
pac

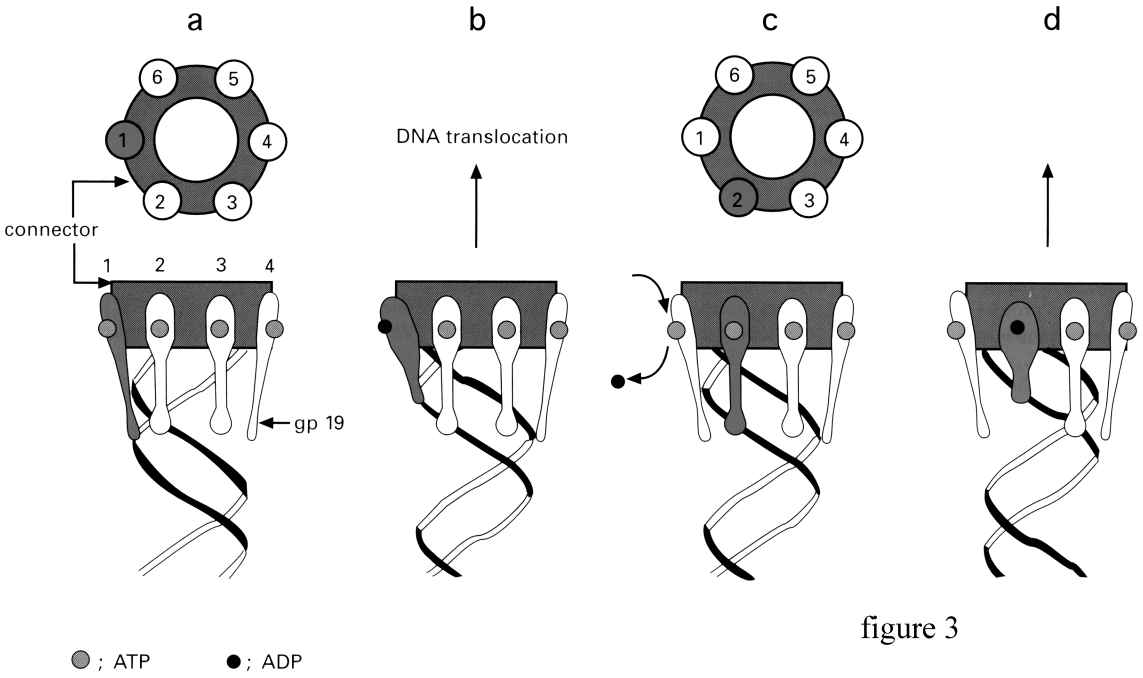


figure 3

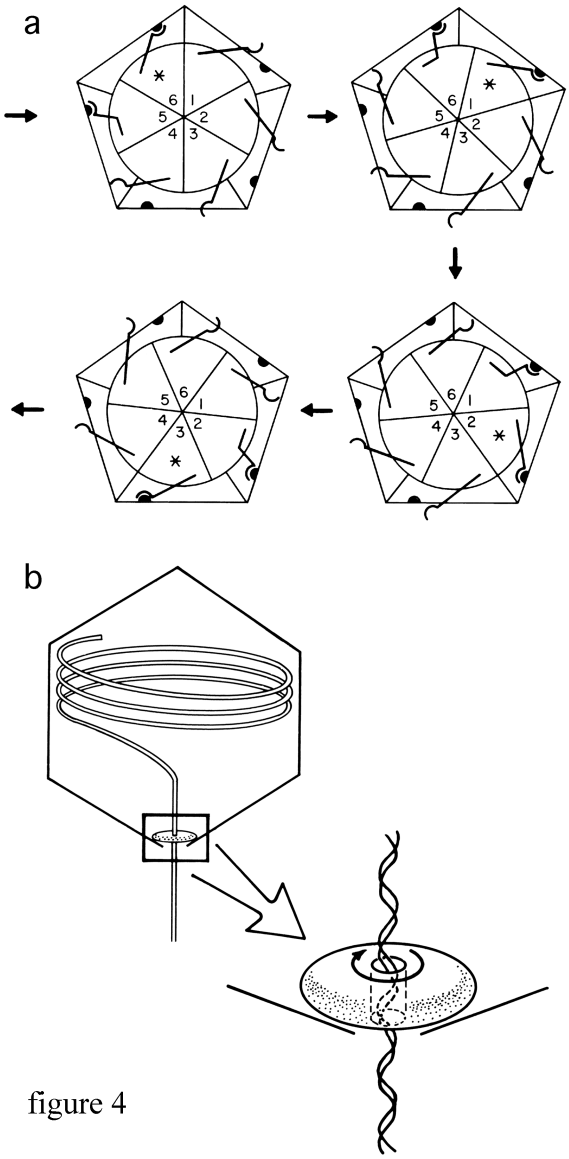


figure 4

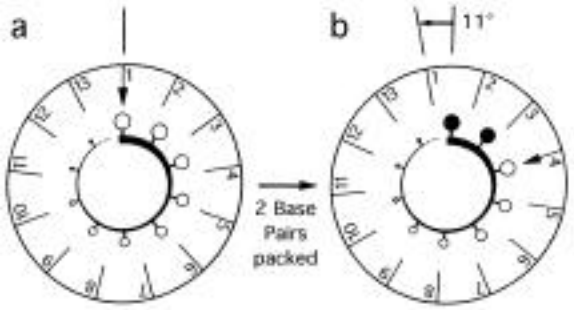


figure 5

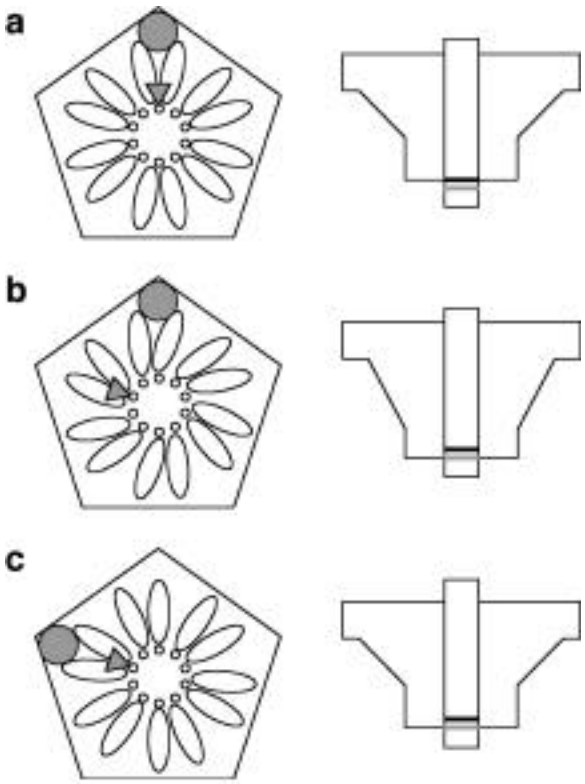


figure 6

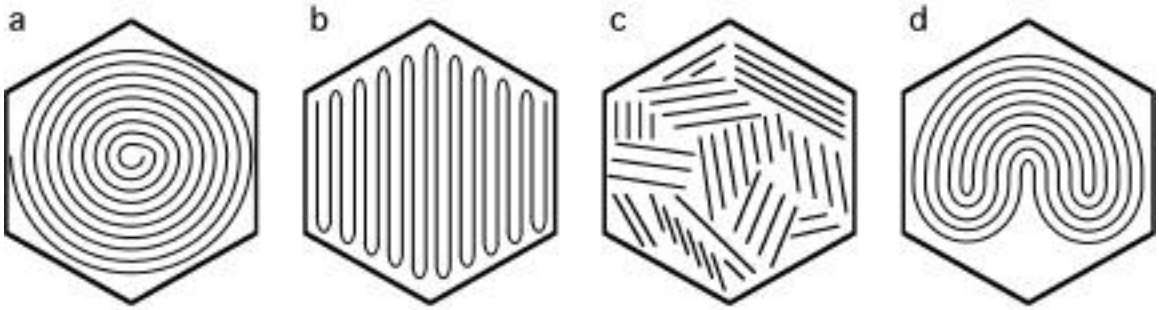


figure 7