CHAPTER 1

Viral Genome Packaging Machines:

An Overview

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A virus particle is a marvel of nature, designed to replicate with a minimal genetic repertoire. All viruses are, of course, obligate intracellular parasites and require an appropriate host in which to develop and multiply. They have evolved a variety of strategies to infect a host cell and to usurp the cellular machinery to manufacture the components required to construct a virion. These precursors are then assembled into an infectious virus particle within the cell.

Virus assembly is a complex process that requires the temporal and coordinated activities of numerous proteins of both viral and host origin. Assembly pathways vary among the virus types, but common features are observed within certain groups. For instance, double-stranded DNA (dsDNA) viruses include the poxviruses, adenovirus, the herpesvirus groups, and many of the bacteriophages. Despite their obvious differences, common development pathways exist among these viruses, as follows. Infection of the host cell ultimately leads to the synthesis of capsid proteins that are assembled into "procapsid" structures. Concurrently, viral DNA is replicated producing numerous copies of the viral genome. The assembly of an infectious virus requires that a single genome be "packaged" into the restricted confines of an empty procapsid. This extraordinary process represents the intersection of the capsid and DNA synthetic pathways, and is an essential step in virus assembly.

This book focuses on the process of viral genome packaging. Chapters 2 through 6 describe our current understanding of genome packaging in bacteriophages λ (Catalano and Feiss), T4 (Black and Rao), T7 (Serwer), P22 (Casjens and Weigele) and SPP1 (Dröge and Tavares). These chapters reveal common mechanisms for DNA packaging among the phage and establish the basic genetic and biochemical rules for the process. In these cases, viral DNA is replicated as linear concatemers of the viral genome. The assembly of an infectious virus requires that individual genomes be cut from the concatemer and concurrently packaged into an empty procapsid, much as one might cut an individual doll from a paper chain and package it into a box. Terminase enzymes are common to these viruses and play a direct role in genome packaging. All of the characterized terminase enzymes are composed of small (18-21 kDa) and large (49-72 kDa) subunits, and the functional holoenzyme is an oligomer of these subunits.

Packaging of viral DNA begins with specific binding of the terminase proteins to a packaging initiation site on the viral DNA concatemer (*pac*, Fig. 1). Specific recognition of viral DNA is mediated by the small terminase subunits. Once assembled, a nuclease activity centered in the large subunit cuts the duplex, thus forming a mature genome end in preparation for DNA packaging. This nucleoprotein complex then binds to a doughnut-shaped portal

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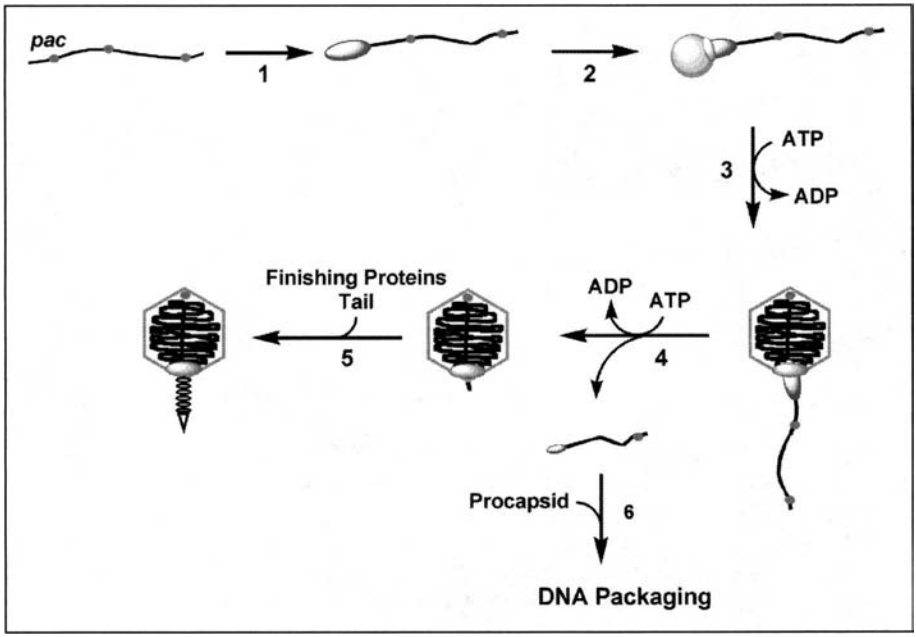

Figure 1. Generalized scheme for genome packaging in double-stranded DNA viruses. A viral genome concatemer is shown as a thick black line with repeated *pac* sites depicted as red dots. The terminase enzyme (blue oval) specifically binds to a *pac* site and cuts the duplex to generate a mature genome end in preparation for packaging (1). The terminase proteins bind to portal proteins located at a unique vertex in a procapsid (cyan sphere with portal shown in purple) (2). This interaction forms the packaging motor that translocates DNA into the capsid, fueled by ATP hydrolysis; DNA packaging triggers capsid expansion (3). Once the entire genome has been inserted into the capsid, terminase again cuts the duplex to complete the packaging process (4). Addition of "finishing" proteins, and a tail in the case of bacteriophages, complete the assembly of an infectious virus (5). The terminase•concatemer complex binds another procapsid to initiate a second round of packaging (6). A color version of this figure is available online at http://www.Eurekah.com.

complex that resides at a unique vertex in the procapsid shell. The portal forms a hole through which DNA enters the capsid during packaging; while details of the interaction remain obscure, it is likely that a combination of the terminase proteins and the portal proteins make up a DNA packaging motor that actively translocates viral DNA into the interior of the capsid. Packaging activity resides in the large terminase subunits and is fueled by ATP hydrolysis.

In many viruses, DNA packaging triggers procapsid expansion. This is a remarkable process where the roughly spherical procapsid undergoes an expansion step that increases the inner capsid volume and yields a more angularized capsid structure. Expansion requires significant reorganization of the capsid proteins and is typically followed by the addition of "stabilization" proteins to the capsid surface, or physical cross-linking of the capsid proteins to provide enhanced structural integrity.

The translocating motor ultimately fills the capsid with DNA, packaging a single viral genome condensed to near liquid crystalline density. This represents an energetically demanding process, and the DNA packaging motor is among the most powerful biological motors thus far characterized. Upon packaging a complete genome, terminase again cuts the duplex, which separates the DNA-filled capsid from the terminase•concatemer complex. The mechanism regulating this terminal cleavage event is unclear, but there is a universal "head-full"

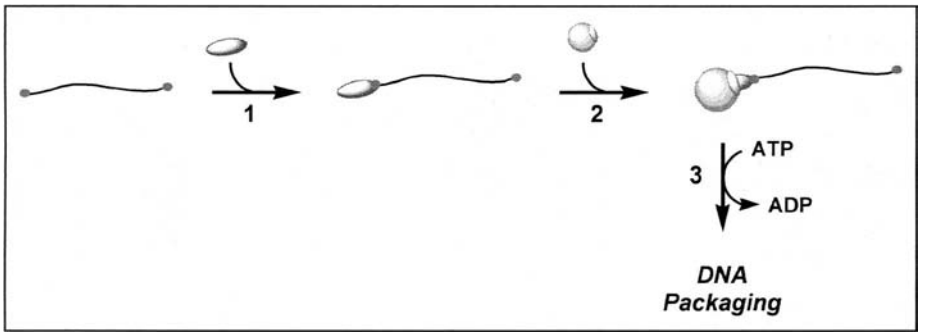

Figure 2. Genome packaging in bacteriophage ϕ29. Viral DNA is replicated as a monomer using a protein primed mechanism. The monomeric genome is shown as a thick black line, with terminal proteins covalently attached to each end (red dots). The terminal protein associates with the "packaging ATPase", shown as a blue oval (1). This complex binds to the portal proteins to complete the packaging motor (2). The motor translocates DNA into the capsid, powered by the hydrolysis of ATP (3). A unique feature of ϕ29 is the requirement for portal-associated RNA molecules in the packaging motor (not shown). A color version of this figure is available online at http://www.Eurekah.com.

component. That is, the nuclease activity of the terminase large subunit is in some way activated once the capsid is filled to capacity. Addition of "finishing" proteins and a viral tail completes the assembly of an infectious virus. The terminase•concatemer complex (described above) binds a second procapsid to initiate a second round of DNA packaging (Fig. 1). Thus, DNA packaging is a processive process, with multiple genomes in the concatemer packaged per DNA binding event.

Not unexpectedly, the details of DNA packaging vary with each virus; however, the similarities are striking and indicate a common strategy. Indeed, this strategy may be universal and traverse the prokaryotic-eukaryotic boundary. The herpesviruses are large eukaryotic dsDNA viruses that encompass many human and animal pathogens. Despite the obvious differences between herpesviruses and bacteriophages, common developmental pathways exist, especially as it relates to DNA packaging mechanisms. Bains and Weller describe our current understanding of herpesvirus genome packaging in chapter nine of this book.

An interesting variation on this general packaging strategy is found in bacteriophage ϕ29, which is described by Anderson and Grimes in Chapter 7 of this book. Unlike the phage described above, ϕ29 replicates its genome as a monomer. This is accomplished through a protein-primed DNA replication mechanism, which yields individual genomes with a terminal protein covalently attached to the 5' ends of the duplex (Fig. 2). Genome packaging requires a "packaging ATPase" protein that associates with the terminal protein. This enzyme also binds to the ϕ29 portal complex to complete the packaging motor. Despite the apparent difference, this mechanism is quite similar to the general packaging strategy, as follows. The small terminase subunits described above provide specific recognition of viral DNA, while the large subunits possess the ATP-powered packaging activity. In the case of ϕ29, the terminal protein (30 kDa) is strictly required for genome packaging and may be viewed as a small terminase subunit. Further, the packaging ATPase of ϕ29 is analogous to the large subunits found in the conventional terminase holoenzymes of λ, T4, etc. The general strategy for genome packaging is thus retained in ϕ29 despite the apparent divergence from the conventional model. Here too, packaging strategies may traverse the prokaryotic-eukaryotic boundary, as packaging in adenovirus may be analogous to that of ϕ29.

The majority of this book examines genome packaging in the dsDNA viruses. In reading these chapters, it becomes apparent that the basic mechanisms of energy transduction linked to DNA translocation are quite similar. This conceptual model is not limited to DNA packaging machines, however. The mechanism of genome packaging in ϕ6, a double-stranded RNA virus, is reviewed in Chapter 8 of this book (Poranen, Pirttimaa and Bamford). In this virus, a ring-shaped NTPase located at a procapsid vertex is responsible for packaging each of three dsRNA segments into the interior of a preformed ϕ6 procapsid. In a twist from the dsDNA viruses, this motor is also responsible for extrusion of newly synthesized message RNAs from the capsid upon the next round of infection. Importantly, the prokaryotic RNA packaging system shows functional similarity to the eukaryotic reoviruses, and again suggests that a general packaging mechanism traverses prokaryotic-eukaryotic boundaries. It is further clear that the ϕ6 packaging and replication machinery share many of the features common to the dsDNA packaging motors.

A coherent mechanistic model for any complex biological process requires (i) a description of the macromolecules involved, (ii) a detailed understanding of how these molecules interact in the formation of larger biological structures, (iii) a description of the catalytic activities associated with these complexes, and (iv) an accounting of the processes that link catalytic activity to structure and function. Genome packaging is a crucial step in virus assembly in a number of prokaryotic and eukaryotic viruses. The molecular motors responsible for this process show mechanistic similarity in viruses as distinct as bacteriophage λ, herpes virus and the dsRNA bacteriophage ϕ6. The chapters in this book provide a detailed summary of our current state of knowledge of the genetics, biochemistry and structure of these fascinating motors. The recent emergence of "new" viral scourges responsible for diseases such as SARS, West Nile fever, etc., and the increasing threat of biological weapons underscore the need to understand virus development at the most basic biological level. We hope that this book provides the experimental background and a philosophical roadmap towards this goal.