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Viral Genome Packaging Machines: Genetics, Structure, and Mechanism

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This book is dedicated to Maria Amparo,
who taught me that the difficult path is likely the most rewarding.

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CHAPTER 1

Viral Genome Packaging Machines:

An Overview

Carlos Enrique Catalano

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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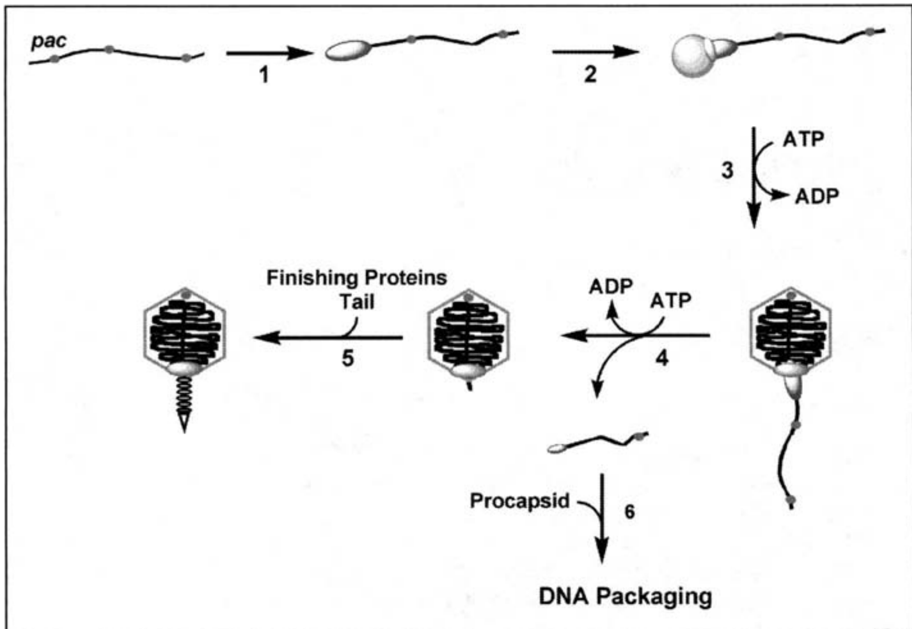

Figure 1. Generalized scheme for genome packaging in double-stranded DNA viruses. A viral genome concatemer is shown as a thick black line with repeated *pac* sites depicted as red dots. The terminase enzyme (blue oval) specifically binds to a *pac* site and cuts the duplex to generate a mature genome 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"

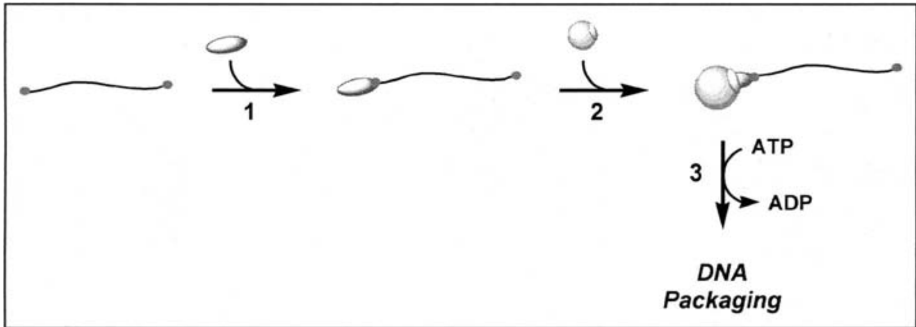

Figure 2. Genome packaging in bacteriophage ϕ29. Viral DNA is replicated as a monomer using a protein primed mechanism. The monomeric genome is shown as a thick black line, with terminal proteins covalently attached to each end (red dots). The terminal protein associates with the "packaging ATPase", 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.

Chapter 2

Bacteriophage Lambda Terminase and the Mechanism of Viral DNA Packaging

Michael Feiss and Carlos Enrique Catalano

Abstract

The developmental pathways of many double-stranded DNA (dsDNA) viruses, both prokaryotic and eukaryotic, are remarkably similar. In viruses as diverse as bacteriophage λ and the herpesviruses, DNA replication proceeds through a rolling circle mechanism where the circular genome serves as a template for the synthesis of linear concatemers multiple genomes in length. Concurrently, viral gene expression produces structural proteins, which self-assemble into procapsids and, in the case of the bacteriophage, tails necessary to assemble an infectious virion. Virus assembly requires that monomeric virion DNA molecules be produced from concatemers during packaging of the DNA into a procapsid. Thus, packaging represents the convergence of the DNA replication and capsid shell assembly pathways. Genome packaging in bacteriophage λ has been extensively studied and this system has been used as a paradigm for virus assembly. Here we summarize current knowledge, present a working model, and indicate issues worthy of further investigation.

Bacteriophage Lambda Infection and DNA Replication

A λ virion consists of a 48.5 kb dsDNA genome tightly packaged within an icosahedral protein shell and a tail, which serves to deliver the linear genome through the cell envelope into the cytoplasm of an *Escherichia coli* cell. Virus infection initiates with adsorption of the virus particle to the surface of a cell; this interaction is mediated by the gpJ[a] protein of the viral tail and the LamB maltodextrin porin protein of the cell (Fig. 1A).[1] A partially understood series of events ultimately leads to "injection" of the genome into the cytoplasm. The linear genome immediately circularizes via 12-base, complementary "sticky" ends and the nicks are sealed by host ligase yielding a circular duplex. The annealed sticky ends form one subsite of *cos*, the cohesive end site of the λ genome.

[a]Proteins expressed from lambda genes are prefaced with a "gp", for gene product. For instance, the protein products of the *J*, *Nu1*, and *A* genes are gpJ, gpNu1, and gpA, respectively.

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Table 1. Genes and proteins involved in lambda assembly

Gene/Sequence	Gene Product	Function
b	gpB	Portal protein
c	gpC	Protease
nu3	gpNu3	Capsid scaffold
e	gpE	Major capsid protein
FI	gpFI	Assembly catalyst
C, E	pX1	gpC-gpE fusion protein, portal
C, E	pX2	gpC-gpE fusion protein, portal
D_L	-	The left end of a mature λ genome
D_R	-	The right end of a mature λ genome
cos	-	DNA sequences required for λ DNA recognition, processing, and packaging into the capsid shell
cosN	-	The *cos* subsite where duplex nicking occurs
cosNL	-	The left *cosN* half-site
cosNR	-	The right *cosN* half-site
cosB	-	gpNu1 binding site for initiation of λ DNA packaging
cosQ	-	The *cos* subsite required for termination of λ DNA packaging
Nu1	gpNu1	The λ terminase small subunit
A	gpA	The λ terminase large subunit
himA, hip	IHF	*E. coli* site specific DNA bending protein
hupA,B	HU	*E. coli* histone-like protein

Bacteriophage λ is a temperate phage, which means that the virus may enter either of two infection pathways. The decision of which pathway to enter depends on the physiology of the host cell and the multiplicity of infection. In the lysogenic pathway, lytic genes are repressed and the viral chromosome integrates into the host chromosome by site-specific recombination, forming a repressed prophage. Lysogeny has been extensively described[2-4] and will not be considered here. The second fate is the lytic pathway.[5] In this case, the lambda *O* and *P* genes are expressed, yielding replication proteins that initiate viral DNA synthesis at *ori*. Initially, DNA synthesis by *E. coli* DNA polymerase III follows a classical Θ replication mechanism where bidirectional replication forks synthesize daughter circles (Fig. 1A). Later during infection, a rolling circle mechanism (σ replication) predominates, which produces linear end-to-end polymers of λ chromosomes, called concatemers. Circular concatemers are also produced by recombination between circular molecules, but linear concatemeric DNA is the major substrate for the assembly of infectious virions.

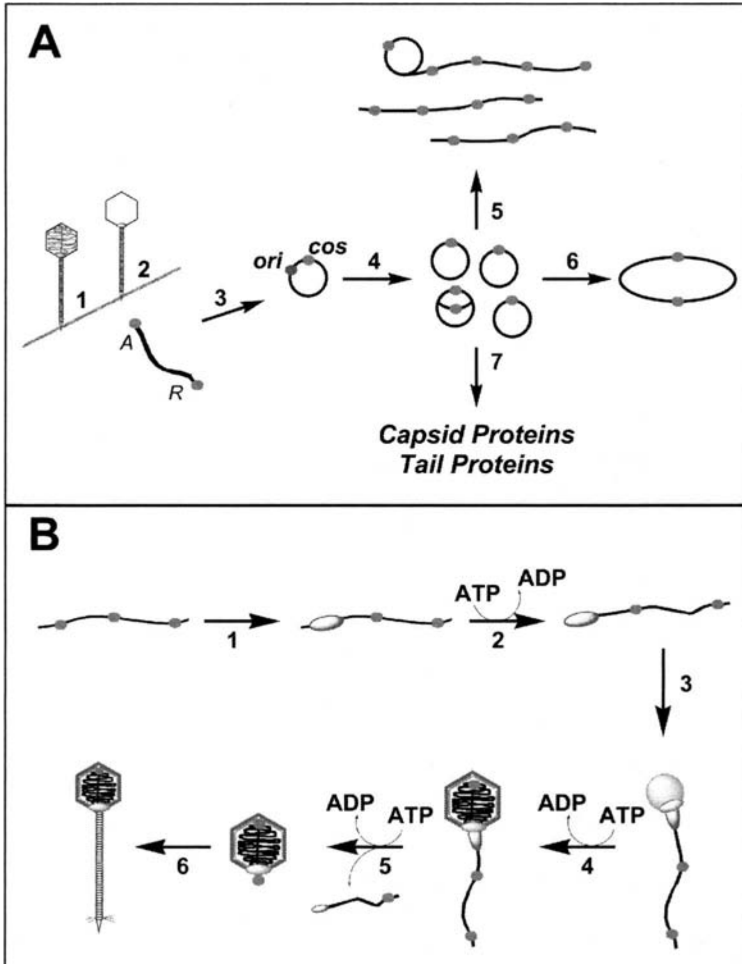

Figure 1. Developmental pathway for bacteriophage lambda. Panel A: Infection of an *E. coli* cell and replication of viral DNA. Infection initiates with adsorption of the virus to the cell surface (1) followed by "injection" of viral DNA into the cytosol of the host cell (2). By convention, the left and right ends of the genome are demarcated by the *A* and *R* genes, respectively. The linear genome circularizes via the 12-base single-stranded cohesive ends (red dots) and the nicks are sealed by host ligase (3). DNA replication initially proceeds via a bi-directional (Θ) replication mechanism yielding daughter circles (4). At later times, DNA is replicated via a rolling circle (σ) mechanism that produces end-to-end concatemers of the viral genome (5). The duplicated *cos* sites in concatemeric DNA are indicated with red dots. Circular concatemers are also produced via recombination (6). Transcription and translation of the viral genome (7) yields structural proteins required to assemble an infectious virus. Panel B: Processing and packaging of viral DNA into a procapsid, and tail attachment to yield an infectious virion. The terminase enzyme (blue oval) binds to a *cos* site in the concatemer (1). The holoenzyme nicks the duplex and separates the strands to "mature" the genome end for packaging (initial *cos*-cleavage) (2). Strand separation requires ATP hydrolysis. The binary terminase•DNA complex binds to a procapsid (cyan sphere) (3) which activates a packaging ATPase activity and translocation of DNA into the capsid (4). DNA packaging promotes capsid expansion, which increases the volume of the capsid and increase angularization of the capsid. The translocating terminase complex stops at the downstream *cos* site and again nicks the duplex (terminal *cos*-cleavage) to complete the packaging process. Strand separation releases the DNA-filled capsid (5) and tail attachment completes the virion (6). A color version of this figure is available online at http://www.Eurekah.com.

recruits a procapsid, sponsors insertion of the DNA into the procapsid, and finally cuts the end of the genome to complete the packaging process (Fig. 1B). As with other terminase enzymes, λ terminase is a heteroligomer composed of small (gpNu1) and large (gpA) subunits (see Fig. 3). GpA carries the DNA cutting activity required to initiate and terminate DNA packaging. This is accomplished through a site-specific endonuclease activity that introduces nicks into *cos* that are staggered by 12 bp. GpA also has a so-called "helicase" activity that separates the nicked strands thus generating the single-stranded "sticky" ends of the mature genome. The gpA subunit further contains a putative DNA translocase activity that is responsible for active DNA packaging, and an ATPase activity that powers translocation. While the large terminase subunit possesses all of the catalytic activities required to cut and package the viral genome, gpA alone exhibits low catalytic activity.[6-10] The gpNu1 subunit specifically recognizes *cos*, and is responsible for the assembly and stability of the packaging machinery. The biological activities of λ terminase are discussed in detail below.

In summary, the packaging pathway entails terminase assembly at a *cos* site in the concatemer and cutting of the duplex (the initial *cos* cleavage reaction), which yields the mature left end of the genome to be packaged. Upon binding a procapsid, the packaging machinery translocates DNA into the capsid through a capsid structure known as the portal vertex (active DNA packaging). Upon arrival at the next downstream *cos* site in the concatemer, the packaging machinery stops and terminase again cuts the duplex generating the mature right end of the genome (the terminal *cos* cleavage reaction); this process yields a single viral genome tightly packaged within the confines of the capsid as described in Figure 1B.

Bacteriophage λ *cos*: A Multipartite Assembly Site

cos

The *cos* site is a ≈200 bp long segment that is required to both initiate and terminate the packaging of a monomeric genome from concatemeric DNA. The site where terminase introduces staggered nicks to generate the cohesive ends is called *cosN* (Fig. 2). Early during the study of λ, it was thought that *cosN* was both necessary and sufficient for DNA packaging. Later studies showed, however, that *cos* is complex and consists of three and perhaps four distinct subsites.[11-15] Both initiation and termination of packaging require duplex nicking at the *cosN* site; additionally, efficient initiation requires the presence of the *cosB* subsite, which is directly downstream from *cosN*. Conversely, efficient termination requires the presence of *cosQ*, a subsite that is located upstream of *cosN* (Fig. 2).[16] The I2 sequence is located between *cosN* and *cosB*, and also plays a distinct role in efficient DNA packaging.[11] Thus, the complete *cos* sequence consists of several subsites, each of which plays a specific role in the recognition, processing, and packaging of viral DNA. Each of these subsites is discussed in detail below.

cosN

The terminase enzyme introduces nicks into the duplex at the *cosN* site to generate the cohesive ends of mature virion DNA. Many of the base pairs (bp) within *cosN* show two-fold rotational symmetry, which extends over 22 bp if one includes purine-purine and pyrimidine-pyrimidine symmetry (Fig. 2); this has been used as evidence that a symmetrically disposed enzyme complex (i.e., a gpA dimer) is responsible for duplex nicking. This argument is further supported by (i) analogies to the interactions of type II restriction endonucleases with their palindromic recognition sequences, and (ii) the presence of a leucine-zipper motif in the primary sequence of gpA.[17] We presume here that a gpA dimer is responsible for symmetric duplex nicking at *cosN*.

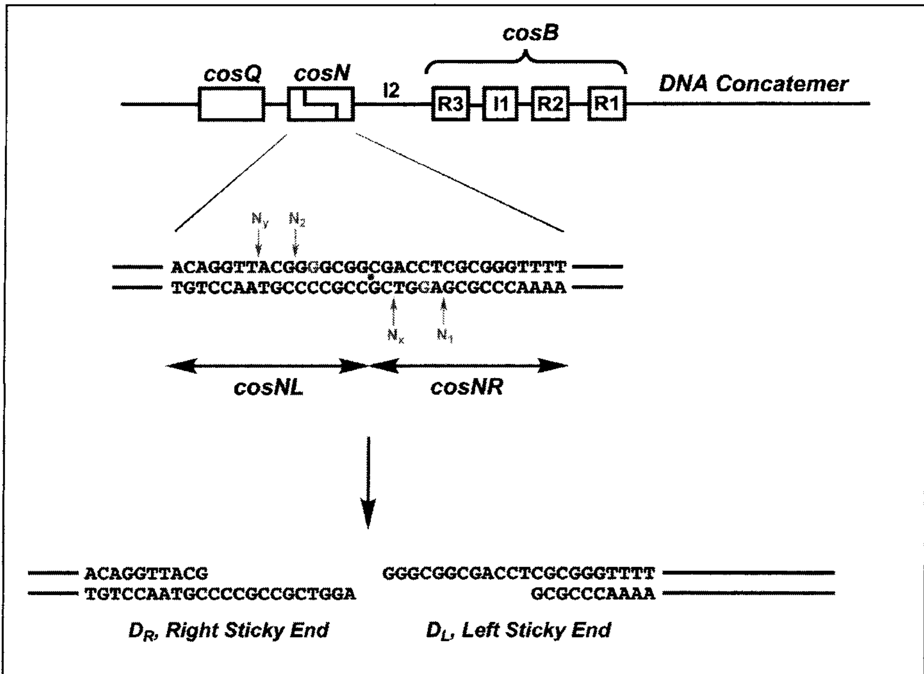

Figure 2. The *cos* region of a lambda concatemer. Upper panel: The *cosQ*, *cosN* and *cosB* subsites within a *cos* site in concatemeric DNA. The *cosB* subsite is composed of the I1 and R-elements, as indicated. The I2 region lies between *cosN* and the R3 element. Middle panel: The nucleotide sequence of *cosN*, with the *cosNL* and *cosNR* half-sites indicated. The center of symmetry of *cosN* is indicated with a dot. Terminase normally nicks the duplex at N_1 and N_2 sites indicated with arrows. In the absence of ATP, terminase incorrectly nicks the duplex at N_x and/or N_y sites. Lower panel: Strand separation by terminase yields the matured D_R and D_L ends of the lambda genome, as shown.

cosB

Terminase nicking of an isolated *cosN* site is error-prone, and the overall rate of nicking is slow.[18,19] The presence of *cosB* and the I2 element (discussed below) is required for efficient and accurate duplex nicking. The *cosB* subsite contains three 16 bp R elements that are specifically recognized by gpNu1 (Fig. 2);[12,33,43,55] specific *cosB*-gpNu1 interactions are likely required to properly position a gpA dimer at *cosN* for the initial *cos* cleavage event.[18,19] The *cosB* subsite also contains a consensus sequence for *Escherichia coli* integration host factor (IHF). This I1 site introduces an intrinsic bend into the duplex, and is also specifically recognized by IHF.[20,21] The role of IHF in DNA packaging and virus assembly is discussed more fully below.

cosQ

This seven base pair subsite is located 17 bp upstream of *cosN* (Fig. 2), and is essential for proper termination of the packaging reaction.[16,22-25] Severe *cosQ* mutations do not significantly affect the initial *cos* cleavage reaction, but abolish nicking of the bottom strand at the terminal *cos* site. Moreover, packaging is not arrested in the absence of *cosQ*, and additional DNA, including the downstream *cos*, is packaged until the capsid shell is filled to capacity.[26] This suggests that *cosQ* is required to stop the packaging machinery for appropriate cleavage at