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Review

# Headful DNA packaging: Bacteriophage SPP1 as a model system

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ABSTRACT

Tailed bacteriophages and herpesviruses package DNA inside the viral capsid by a powerful molecular motor. This packaging machine is composed of the portal protein, which provides a gate for DNA entry, the large terminase subunit whose ATPase activity fuels DNA translocation, and most frequently, a small terminase subunit that recognizes the viral packaging site. Here we review the mechanisms how the virulent *Bacillus subtilis* phage SPP1 packages DNA into a preformed procapsid. Encapsidation of the SPP1 DNA follows a processive unidirectional headful mechanism that starts with the recognition and cleavage of a unique genomic sequence (*pac*) by the viral terminase. The viral genome is then translocated through the central channel of the portal protein found at a single vertex of the procapsid. Packaging is terminated by an endonucleolytic cleavage of the concatemeric DNA substrate, following by disassembly of the packaging motor and closure of the portal system by the gatekeepers preventing leakage of the viral genome. Recent advances are providing new molecular insights on the mechanisms that ensure precise coordination of these critical steps required to accomplish the packaging encapsidation cycle.


**Contents**



*Abbreviations:* DBD, DNA binding domain; dsDNA, double-stranded DNA; bp, base pairs; CTD, C-terminal domain; gpx, gene product X; HTH, helix-turn-helix domain; LT, large terminase subunit; *PL1*, late promoter 1; *PL2*, late promoter 2; *pacC*, gp2 cleavage subsite; *pacL*, non-encapsidated gp1 recognition site; NTD, N-terminal domain; *pacR*, encapsidated gp1 recognition site; ST, small terminase subunit; wt, wild type.

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## 1. Introduction[1]

A fundamental step in the life cycle of tailed bacteriophages (or phages) and herpesviruses is the encapsidation of their dsDNA genome into a preformed icosahedral procapsid. DNA packaging in these viruses requires assembly of a complex molecular machine at a specific vertex of the icosahedral procapsid. This vertex is characterized by the presence of the portal protein, a ring-like oligomer possessing a central channel through which DNA translocation occurs (Bazinet and King, 1985; Casjens and Hendrix, 1988; Valpuesta and Carrascosa, 1994; Cuervo and Carrascosa, 2012). Initiation of packaging involves the specific recognition and binding of the viral terminase complex to a specific region of phage dsDNA. The substrate DNA is a head-to-tail multimer of the viral genome (concatemer) in most phage species, resulting from viral genome replication (Lo Piano et al., 2011). The terminase is normally composed of two subunits, a small terminase (ST) subunit involved in viral DNA recognition, and a large terminase (LT) subunit possessing endonucleolytic and ATPase activities. The DNA-terminase nucleoprotein complex then interacts with the portal vertex and DNA translocation begins, fuelled by the ATPase activity of the terminase (Yeo and Feiss, 1995; Lin et al., 1999; Oliveira et al., 2010). Packaging is normally terminated by an endonucleolytic cleavage of the concatemeric DNA used as substrate for encapsidation by most phages (Black, 1989).

Two general modes for cleavage and packaging of the viral concatemeric dsDNA have been described. In the first one (phages like λ, T3 and T7) packaging is initiated and terminated by a specific cut at a unique and precise dsDNA sequence (termed *cos* in phage λ), generating unit-length encapsidated molecules (Catalano et al., 1995; Fujisawa and Morita, 1997). In the second mode (phages like T4, P22, SPP1, P1, T1), headful sized DNA segments are sequentially packaged from the concatemeric DNA precursor (Black, 1989; Tavares et al., 1996). Encapsidation initiates most often by cleavage at a specific sequence in the genome termed *pac* (P22, SPP1, P1, T1). The level of DNA head-filling then triggers a sequence-independent cleavage of the substrate DNA concatemer defining the size of the packaged DNA. At the end of the encapsidation cycle the terminase, bound most probably to the DNA extremity generated by the termination endonucleolytic cleavage, leaves the portal vertex. This nucleoprotein complex then interacts with the portal vertex of another procapsid and a new encapsidation cycle initiates (processive packaging). The sequential headful packaging mechanism generates a heterogeneous population of terminally redundant and circularly permuted DNA molecules. The SPP1, P22 and P1 phages use a *pac* sequence to initiate the DNA packaging series and permutation accounts up to 25% of the genome (Tye et al., 1974; McIntosh et al., 1978; Morelli et al., 1979; Tavares et al., 1996; Sternberg and Coulby, 1987). Depending of the length of the concatemeric DNA substrate partially or complete circular permutations are generated. Phage T4 is similar to *pac* phages except that the first cut does not strictly occur within a unique *pac* sequence. It was proposed, nevertheless, that at least one preferential sequence may be recognized by the T4 ST subunit (Rao and Black, 2005). However, initiation of packaging shall start at different regions of the T4 genome as this phage shows complete circular permutation (Streisinger et al., 1967).

The virulent *Bacillus subtilis* siphovirus SPP1 is a well-established model system for phages that package their DNA by a headful mechanism (Dröge and Tavares, 2000, 2005; Oliveira et al., 2005). Encapsidation is initiated by recognition and cleavage by the terminase of the nucleotide sequence *pac* followed by unidirectional translocation of a concatemeric DNA to the interior of the procapsid. When a threshold amount of DNA, representing about 103% of the SPP1 genome, has been packaged (headful) a sequence-independent DNA cleavage terminates the encapsidation cycle (Fig. 1A). The goals of this article are to review recent progress on the understanding of the molecular mechanism used by SPP1 to package its DNA, and to provide an integrated view of our current knowledge of the headful DNA packaging process.

## 2. Assembly of the SPP1 procapsid, the container that accommodates the viral genome

SPP1 devotes more than 40% of its genetic information to the synthesis and assembly of structural components (Alonso et al., 2006). The phage capsid and the tail are formed in independent assembly pathways. The sequence of reactions that yields the SPP1 viral particle was studied in detail (Fig. 1B). The DNA-filled capsid that binds to preformed tails is composed of 6 viral proteins (gp6, gp7, gp12, gp13, gp15 and gp16; Table 1). Its assembly and packaging of the viral genome requires three non-structural SPP1 proteins (gp11, gp1 and gp2). The role of host proteins in assembly was not studied.

The first detectable intermediates of capsid assembly are spherically shaped procapsids with an outer diameter of ~55 nm. These are composed of the portal protein gp6 (57.3 kDa), the minor capsid protein gp7 (35.1 kDa), the scaffolding protein gp11 (23.5 kDa) and the major capsid protein gp13 (35.4 kDa) (Fig. 1B; Alonso et al., 1997; Becker et al., 1997; Dröge et al., 2000a,b). The procapsid icosahedral lattice has a $T=7$ symmetry. It is formed by 415 subunits of the major capsid protein gp13 that has a fold similar to the one described for the phage HK97 major capsid protein (Dröge et al., 2000a,b; White et al., 2012). The interior of the procapsid is "filled" with 100–180 copies of the scaffolding protein gp11. Gp11 is an α-helical-rich elongated molecule, as found for internal scaffolding proteins from different tailed phages (Poh et al., 2008; Prevelige and Fane, 2012). Unlike other scaffolding proteins that are most frequently dimers, gp11 is a tetramer formed of a dimer of dimers in solution. This species is able to direct polymerisation of gp13 synthesized *in vitro* to assemble correctly shaped procapsid-like structures (Poh et al., 2008). It remains to be established if gp11 is a tetramer in the assembled procapsid or whether this oligomeric state is a storage form that dissociates into dimers during the procapsid building process (Poh et al., 2008). No interaction was observed between gp11 and gp13 when the two proteins are produced independently and then mixed *in vitro* suggesting that their assembly occurs co-translationally (Becker et al., 1997; Dröge et al., 2000a,b). This hypothesis is further supported by the observation that co-production of gp11 and gp13 yields procapsid-like structures both in *B. subtilis* infected with a SPP1 phage carrying nonsense mutations in genes 1 and 6 (coding respectively for the ST subunit and the portal protein) or when genes *11* and *13* are co-expressed in *Escherichia coli* from a plasmid (Becker et al., 1997; Dröge et al., 2000a,b). In both conditions the procapsid-like structures composed of gp11 and gp13 are a mix of particles with the normal procapsid size ($T=7$) and others with a smaller dimension ($T=3$ or 4). None of these are competent for viral DNA encapsidation (Dröge et al., 2000a,b). The third component required for biological activity of the SPP1 procapsid is gp6 that is found at one of the twelve vertices of the icosahedral procapsid. The ring-like structure of the gp6 12-mer defines a specialised vertex for phage DNA packaging (Fig. 1B). Presence of gp6 correlates with predominant formation of $T=7$ procapsids showing that the portal protein influences the co-polymerisation of gp11 and gp13 preventing assembly of small $T=3$ or 4 procapsids (Dröge et al.,

[1] Note that unless otherwise, the indicated genes and products are of *B. subtilis* phage SPP1 or its relative SF6 origin. In this review we have used the gpX nomenclature that is synonymous to GXP used in other publication on SPP1 or SF6 research.