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Review

Old, new, and widely true: The bacteriophage T4 DNA packaging mechanism

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ABSTRACT

DNA packaging into empty viral procapsids by ATP-driven motor proteins applies widely among viruses. Recent fluorescence studies of phage T4 reveal: 1) the small terminase subunit (TerS) synapses *pac* homologs by a twin ring mechanism to gauge DNA maturation and allow packaging by the large terminase subunit (TerL); 2) translocation of linear DNA is efficient by TerL acting alone; expansion of the procapsid is controlled by the portal-terminase assembly; 3) both ends of the packaged DNA are held at the portal, showing a loop of DNA is packaged; 4) transient spring-like compression of B form to A form-like DNA accompanies translocation; 5) the C-terminal domain of TerL is docked to the portal and moves toward it when stalled; 6) a portal bound resolvase can release stalled Y-DNA compression and allow translocation in vitro; and 7) ATP powered translocation on A form dsDNA is supported by recent hexameric helicase studies.

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Following the early demonstration (using DNA packaging essential gene 49:EndoVII (Holliday junction resolvase) mutants) that prohead filling could be completed in bacteriophage T4 infected bacteria (Luftig et al., 1971), diverse mechanisms were proposed to explain prohead filling to high DNA density. Among others, DNA replication, prohead expansion, DNA binding to prohead internal proteins, and scaffold-core protein proteolysis and exit were proposed to drive packaging energetically. Despite attractive features of coupling DNA packaging to these aspects of phage development in vivo (Fig. 1), these proposed mechanisms were shown not to apply. For example, the phage T4 internal proteins found with the DNA in the filled bacteriophage capsid are dispensable for packaging, and, as generally found to be the case where tested, these packaged proteins enter the prohead before the DNA. Most are then ejected along with the DNA into the host for early roles in infection; surprisingly, large and multimeric proteins packaged into the early precursor prohead scaffold or core can pass through a narrow diameter portal and tail tube channel into the host (Black and Thomas, 2012).

Bacteriophage T4 was the first phage shown to package its DNA by a terminase motor docked to a procapsid portal. Early structural studies revealed a unique vertex ring dodecamer structure that could be extracted from the prohead (Muller-Salamin et al., 1977). Order of function studies (Jarvik and Botstein, 1973) showed that the T4 portal had an early essential function in assembling an empty prohead and then a late essential function in packaging coupled to the terminase-ATPase motor (Hsiao and Black, 1977). A torsional DNA compression terminase-portal motor was proposed to use gyrase-like DNA supercoiling to drive packaging by energizing the DNA (Black and Silverman, 1978). An alternative early hypothetical portal-centric mechanism based on a symmetry mismatch at the portal vertex proposed a rotary portal motor; this model was long favored, possibly in part as rotary motor mechanisms were in fact demonstrated for ATP synthase and the bacterial flagella motor (Hendrix, 1978; Simpson et al., 2000). Only comparatively recently has rotation of the portal been shown *not* to be a feature of the motor mechanism in T4 (Baumann et al., 2006) (Fig. 2) or in φ29 (Hugel et al., 2007). Instead two linear

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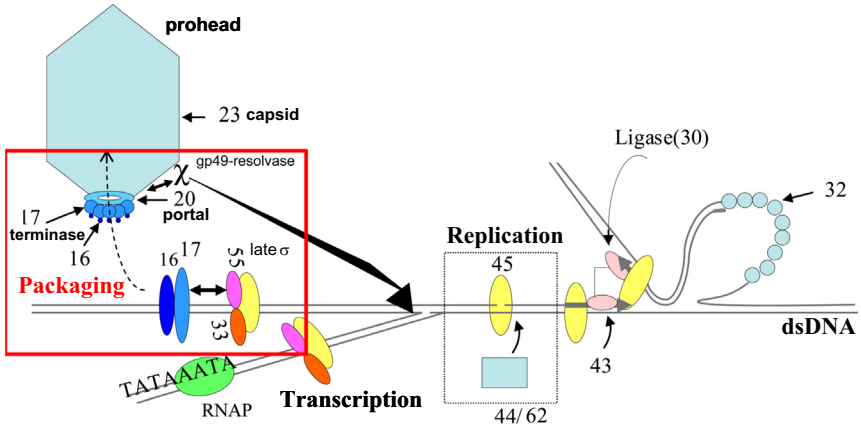

Fig. 1. Regulatory integration in vivo of packaging, late transcription, and replication among T4-type phages. The phage T4 terminase large subunit ATPase motor protein gp17 (TerL) and the small terminase DNA recognition protein gp16 (TerS) initiate packaging. A gp20 portal bound packaging essential gp49:Endonuclease VII resolvase removes X and Y structures blocking packaging on a branched DNA concatemer in vivo. The gp55 late sigma factor interacts with TerL and appears to be essential for initiating concatemer packaging.

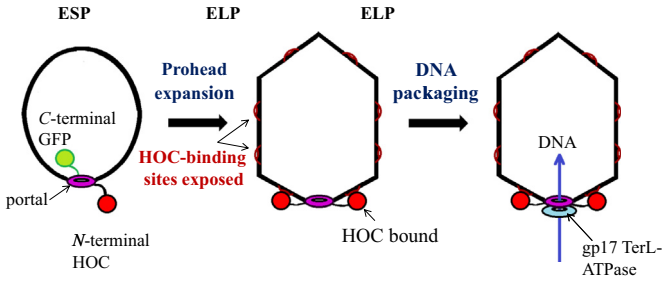

Fig. 2. Evidence against a proposed prohead portal rotory motor mechanism. No rotation of six C-terminal portal-GFPs found as part of the portal dodecamer inside filled 500 mg/ml DNA active phages; no rotation of N-terminal HOC-gp20 portal protein tethered to HOC binding sites on expanded mature in vitro packaging active proheads. ESPs are immature empty small proheads, ELPs are mature and stable empty large proheads with HOC decoration protein binding sites exposed (Baumann et al., 2006).

terminase motor mechanisms for the T4 packaging motor were proposed simultaneously (Oram et al., 2008; Sun et al., 2008).

Many years of experimental study have supported the basic similarity of phage and viral DNA packaging motor mechanisms. The similar high resolution structures of proheads, portals, and large terminase subunits (TerL) support this view. Packaging is regulated differently in different phages; e.g. bacteriophage T4 packaging in vivo is connected to the DNA sliding clamp-late sigma gp55 complex (Black and Peng, 2006; Malys et al., 2002), tying it to both late transcription (Geiduschek et al., 1997) and replication. This interaction likely promotes TerL binding to the concatemeric DNA or to DNA repair, and this role may be similar to the essential packaging role of T7 and T3 RNAPs (Fig. 1). However T4 terminase small subunit gp16 (TerS) is essential in vitro and in vivo for packaging of circular or concatemeric DNA (i.e., DNA with no or few ends), the latter being the in vivo substrate that is subject to complex regulation of packaging initiation among *pac* site phages (Fig. 3) (Black and Peng, 2006). In *pac* site phages the TerS is required to initiate packaging at specific *pac* sites on concatemeric DNA by "handing off" the DNA to the large nuclease-containing terminase subunit (TerL) for packaging initiation cutting. In contrast, T4 TerL acting alone can package in vitro linear DNA with high efficiency.

Substantial evidence supports a double TerS protein ring double DNA *pac* site "synapsis model" as a mechanism to assess DNA concatemer maturation (Black, 1995) (Fig. 3). There is both genetic and biochemical evidence for this proposed mechanism for initiation of phage T4 packaging: i) purified TerS displays enhanced binding to a GC-rich sequence at the 3′ end of its gene that was identified as a *pac* site (Lin et al., 1997): ii) this sequence confers enhanced transduction of phage and plasmid DNAs containing it in vivo by a transducing derivative of T4 (Lin and Black, 1998); iii) under selection for increased synthesis of TerL the TerS protein is shown to be required for gene amplifications requiring recombination between homologous gene *16* and *19 pac* sequences (see Fig. 3) (Wu et al., 1995). Such sequence specific amplifications require a phage internal alt protein knock-out to allow substantially more DNA to be packaged into the phage particle, apparently by increasing head volume, thus allowing multiple copies of the *16–19* region to be viable within the chromosome (Wu and Black, 1987; Wu et al., 1991); iv) a mature DNA restriction fragment containing a gene 16 *pac* site can be found in phage particles (Lin and Black, 1998); however site directed mutagenesis of the gene 16 amplification *pac* site that eliminates gene 16–19 amplifications is not lethal to the phage, suggesting backup *pac* sites (such as the homologous 19 *pac* site (see Fig. 3B) or other mechanisms (Wu and Black, 1995); and finally v) mass spectrometry and scanning transmission electron microscopy mass determination show single 11mer and double 22mer rings. These are proposed to be aplanar, lock washer like single- and double-ring structures that unstack to yield side-by-side rings (Lin et al., 1997). The single and double rings purified from an untagged protein expression vector are stable by mass spectrometry and have been shown to contain only protein (van Duijn, 2010). The two rings thus are not held together with DNA as hypothesized for another proposed two-ring TerS structure, although without direct experimental support (Sun et al., 2012). Unlike crystal structures of His-tagged TerS that by mass form 11mers and 12mers, the purified untagged rings are by mass only 11mers (not 12mers) and 22mers. Of course in vivo the TerS protein is expected to interact with DNA, possibly as shown in Fig. 3C.

An unexpected difference between TerL and TerS is that while the large terminase has long been known to be a signature homology feature among diverse phages (Black, 1989) [for informatics see Fig. 2 in Serwer and Jiang (2012)], the small terminase genes and gene product crystal structures display marked differences. Moreover major controversies abound as to how these crystal structures relate to TerS DNA binding in various *pac* site phages. Thus whether the *pac* DNA binding-site is at the N-terminus of TerS (where there is generally a DNA binding motif) or at its C-terminus, and how this relates to number of monomers per crystal ring structure (8, 9, 10, 11, and 12 per single ring have been reported among different phages), as well as the very different TerS monomer structures [for a gallery of the single ring