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Novel DNA packaging recognition in the unusual bacteriophage N15

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ABSTRACT

Phage lambda's *cosB* packaging recognition site is tripartite, consisting of 3 TerS binding sites, called R sequences. TerS binding to the critical R3 site positions the TerL endonuclease for nicking *cosN* to generate cohesive ends. The N15 *cos* (cos^{N15}) is closely related to cos^{λ}, but whereas the $cosB^{N15}$ subsite has R3, it lacks the R2 and R1 sites and the IHF binding site of $cosB^{\lambda}$. A bioinformatic study of N15-like phages indicates that $cosB^{N15}$ also has an accessory, remote rR2 site, which is proposed to increase packaging efficiency, like R2 and R1 of lambda. N15 plus five prophages all have the rR2 sequence, which is located in the TerS-encoding *1* gene, approximately 200 bp distal to R3.

An additional set of four highly related prophages, exemplified by Monarch, has R3 sequence, but also has R2 and R1 sequences characteristic of cosB–λ. The DNA binding domain of TerS-N15 is a dimer.

Introduction[6]

Large DNA viruses, such as tailed bacteriophages and herpes viruses, use an ATP-powered motor to translocate viral DNA into the preformed empty shell, called the prohead or procapsid (Catalano, 2005; Newcomb et al., 1999; Rao and Feiss, 2008). Recent structural and bioinformatic studies demonstrate that the DNA packaging machinery of these viruses is descended from that of an ancient common ancestor. For example, the prohead shell is an icosahedral lattice principally constructed of many copies of a major capsid protein whose fold is conserved (Baker et al., 2005). Similarly, one of the prohead's 5-fold vertexes, the unique portal vertex, contains the radially disposed dodecameric portal protein. The portal protein contains a channel through which DNA enters and exits the shell interior (Agirrezabala et al., 2005; Lebedev et al., 2007; Simpson et al., 2000; Trus et al., 2004). Terminase, also conserved in the herpes viruses and tailed bacteriophages, is usually a hetero-oligomer of a small and a large subunit (Casjens, 2011; Feiss and Rao, 2012; Przech et al., 2003). The terminase small subunit (TerS) carries out viral DNA recognition. The large subunit contains the ATPase center that powers

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[6] Abbreviations and nomenclature; IHF=integration host factor. wHTH=winged helix-turn-helix DNA binding motif. TerS and TerL denote the terminase small and large subunits, respectively. Hybrid phages, derivatives of λ carrying cos and terminase gene segments from N15 are designated by isolation numbers, for example, $N15^{hy4}$. The two segments of recombinant genes of chimeric phages are denoted by both gene names and the hybrid identity: the small terminase subunit of λ $N15^{hy4}$ is $1/Nu1^{hy4}$; the gene product is $gp1/Nu1^{hy4}$. Sequence convention: for both λ and N15, bp numbers start with the first base of the left cohesive end.

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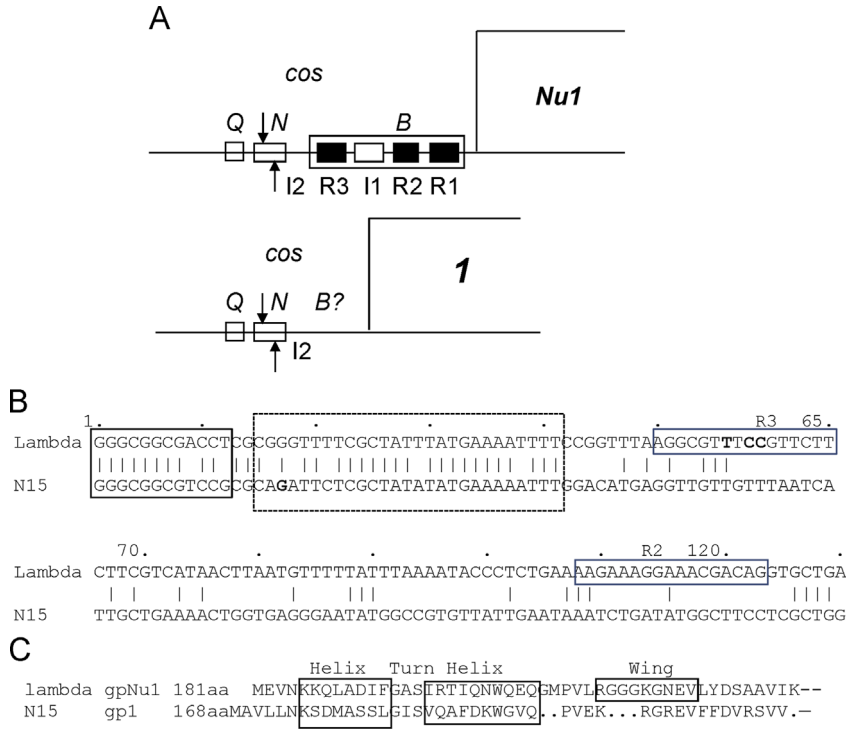

Fig. 1. Comparison of the *cos*es and terminase small subunit genes of λ, 21 and N15. (A) cos^{λ} (above) and cos^{N15} (below). (B) Alignment of left ends of λ (above) and N15 (below) DNAs. Vertical lines indicate sequence identity. Bp 1–15 (boxed) are *cosN* base pairs at the mature left chromosome ends. The large box (dotted) is I2, a sequence feature highly conserved in λ-like phages. The R3 and R2 segments of cos^{λ} are also boxed. (C) Alignment of the DNA binding segments of the λ and N15 terminase small subunits. The winged helix-turn-helix DNA binding motif of λ's gpNu1 is formed by residues 5–12 (support helix), 13–15 (turn), 16–25 (recognition helix) and wing (residues 31–39) (de Beer et al., 2002).

translocation of the DNA into the prohead and the endonuclease that cuts the concatemeric DNA into unit-length virion chromosomes.

For temperate bacteriophage λ, viral DNA packaging occurs in a cell containing roughly equal amounts of phage and host DNA. Virion λ chromosomes, produced during the packaging process, are linear dsDNA molecules with cohesive ends, i.e., complementary, 5′-ended, 12 base-long, single-strand extensions that anneal, circularizing the DNA, upon entry into a host cell. The DNA segment containing the annealed cohesive ends is called *cos*. At late times during the lytic cycle, recombination and rolling circle replication produce concatemeric DNA (Furth and Wickner, 1983). During packaging, concatemeric DNA is recognized and processed by terminase into monomeric virion chromosomes. The small and large subunits of λ terminase are gpNu1 and gpA, the gene products of the *Nu1* and *A* genes, respectively. The simplest form of λ terminase is the protomer, a $gpNu1_2$:gpA_1 heterotrimer. Protomers further assemble into tetramers of heterotrimers (Maluf et al., 2006, 2005). GpNu1's N-terminal domain, which specifically binds *cos*, is a tight dimer containing a winged helix-turn-helix (wHTH) DNA binding motif (de Beer et al., 2002; Feiss et al., 2010). GpA contains two large domains: the N- and C-terminal halves contain the translocation ATPase and the cohesive end-generating endonuclease, respectively (Duffy and Feiss, 2002; Hang et al., 2001; Hwang and Feiss, 2000; Ortega et al., 2007). GpNu1 recognizes λ DNA by specific binding to *cosB*, a *cos* subsite adjacent to *cosN*, where gpA endonuclease domains introduce staggered nicks to generate the cohesive ends (Davidson and Gold, 1992; Hang et al., 2001; Higgins and Becker, 1995; Higgins et al., 1988; Hwang and Feiss, 2000). *cosB* binding by gpNu1 properly positions gpA endonuclease domains on *cosN* for introducing nicks. When *cosB* is deleted or re-positioned, *cosN* nicking is inefficient and inaccurate (Hang et al., 2001) Higgins (Higgins and Becker, 1994a, 1995; Miller and Feiss, 1988).

Packaging is initiated when terminase binds and nicks a *cos* of a concatemer. Following *cosN* nicking and cohesive end separation, terminase forms a tight complex, Complex I, on the *cosB*-containing chromosomal end (Becker et al., 1977; Yang et al., 1997). Complex I then docks on the portal protein, gpB, of the prohead (Dokland and Murialdo, 1993; Lander et al., 2008). Following portal docking of Complex I, gpA's ATPase is activated and ATP hydrolysis-powered translocation of the DNA into the shell ensues (Dhar and Feiss, 2005; Yang and Catalano, 2003). Terminase remains docked on the portal during translocation. When the next *cos* along the concatemer is encountered, terminase (1) nicks *cosN*, (2) dissociates from the portal, and (3) remains bound to the *cosB*-containing end of the next chromosome along the concatemer (Feiss et al., 1985). By remaining bound to the next chromosome along the concatemer as a Complex I, terminase continues to package downstream chromosomes in a processive manner. The third *cos* sub-site, *cosQ*, is essential for recognition and nicking of the downstream *cos* during termination of packaging (Cue and Feiss, 1997; Wieczorek and Feiss, 2001). In sum, *cosN* and *cosB* are required to initiate λ DNA packaging, *cosQ* and *cosN* are required for termination, and all three subsites are required for processivity.

The 120 bp-long $cosB^{\lambda}$ is complex, containing three gpNu1 binding sites, R3, R2, and R1 (Fig. 1) (Catalano et al., 1995). Between R3 and R2 is a binding site, I1, for IHF, the *Escherichia coli* bending protein (Bear et al., 1984; Kosturko et al., 1989; Ortega and Catalano, 2006; Xin and Feiss, 1993). IHF bends DNA into an approximately 180° hairpin (Rice et al., 1996). At *cos*, the IHF-induced I1 bend positions R3 and R2 such that the wHTH motifs of dimeric gpNu1 can be docked into the major grooves (de Beer et al., 2002). Complex I likely includes this nucleoprotein structure (Yang et al., 1997).

Phage 21 is a λ-like phage whose head genes share strong sequence identity with λ's head genes – the two phages are descended from a common ancestor phage, as follows. The