Structure–Function Analysis of the DNA Translocating Portal of the Bacteriophage T4 Packaging Machine

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Abstract

Tailed bacteriophages and herpesviruses consist of a structurally well conserved dodecameric portal at a special 5-fold vertex of the capsid. The portal plays critical roles in head assembly, genome packaging, neck/tail attachment, and genome ejection. Although the structures of portals from phages ϕ29, SPP1, and P22 have been determined, their mechanistic roles have not been well understood. Structural analysis of phage T4 portal (gp20) has been hampered because of its unusual interaction with the *Escherichia coli* inner membrane. Here, we predict atomic models for the T4 portal monomer and dodecamer, and we fit the dodecamer into the cryo-electron microscopy density of the phage portal vertex. The core structure, like that from other phages, is cone shaped with the wider end containing the "wing" and "crown" domains inside the phage head. A long "stem" encloses a central channel, and a narrow "stalk" protrudes outside the capsid. A biochemical approach was developed to analyze portal function by incorporating plasmid-expressed portal protein into phage heads and determining the effect of mutations on head assembly, DNA translocation, and virion production. We found that the protruding loops of the stalk domain are involved in assembling the DNA packaging motor. A loop that connects the stalk to the channel might be required for communication between the motor and the portal. The "tunnel" loops that project into the channel are essential for sealing the packaged head. These studies established that the portal is required throughout the DNA packaging process, with different domains participating at different stages of genome packaging.

Introduction

Tailed bacteriophages and herpesviruses use powerful molecular machines to package their genomes into a head or a capsid. The packaging machine consists of two basic components: a portal through which DNA genome enters the capsid and a motor that drives DNA translocation fueled by ATP (Fig. 1) [1–3].

The heads of phage T4 are assembled on the membrane. The portal of T4 (gp20) and of other phages is a dodecamer [4,5]. It is the first structure assembled in the head assembly pathway (Fig. 1a). The portal nucleates the assembly of the hexameric capsomers each composed of six copies of the major capsid protein (gp23) into capsids. The portal also nucleates the assembly of the major scaffolding protein (gp22). Together, these interactions lead to the formation of the first 5-fold vertex of the icosahedral capsid [6–8]. It also creates a symmetry mismatch between the dodecameric portal and the fivefold capsid, a feature strictly conserved in all well-characterized tailed phages and herpesviruses. Head assembly continues by co-polymerization of the capsid protein and the scaffolding proteins (gp21, gp22, gp67, gp68, IPI, IPII, IPIII, and gpAlt) to form a "prehead" (Fig. 1b). A unique feature of phage T4 is that its portal assembles on the *Escherichia coli* inner membrane, assisted by the membrane-bound phage chaperone gp40 and the *E. coli* membrane

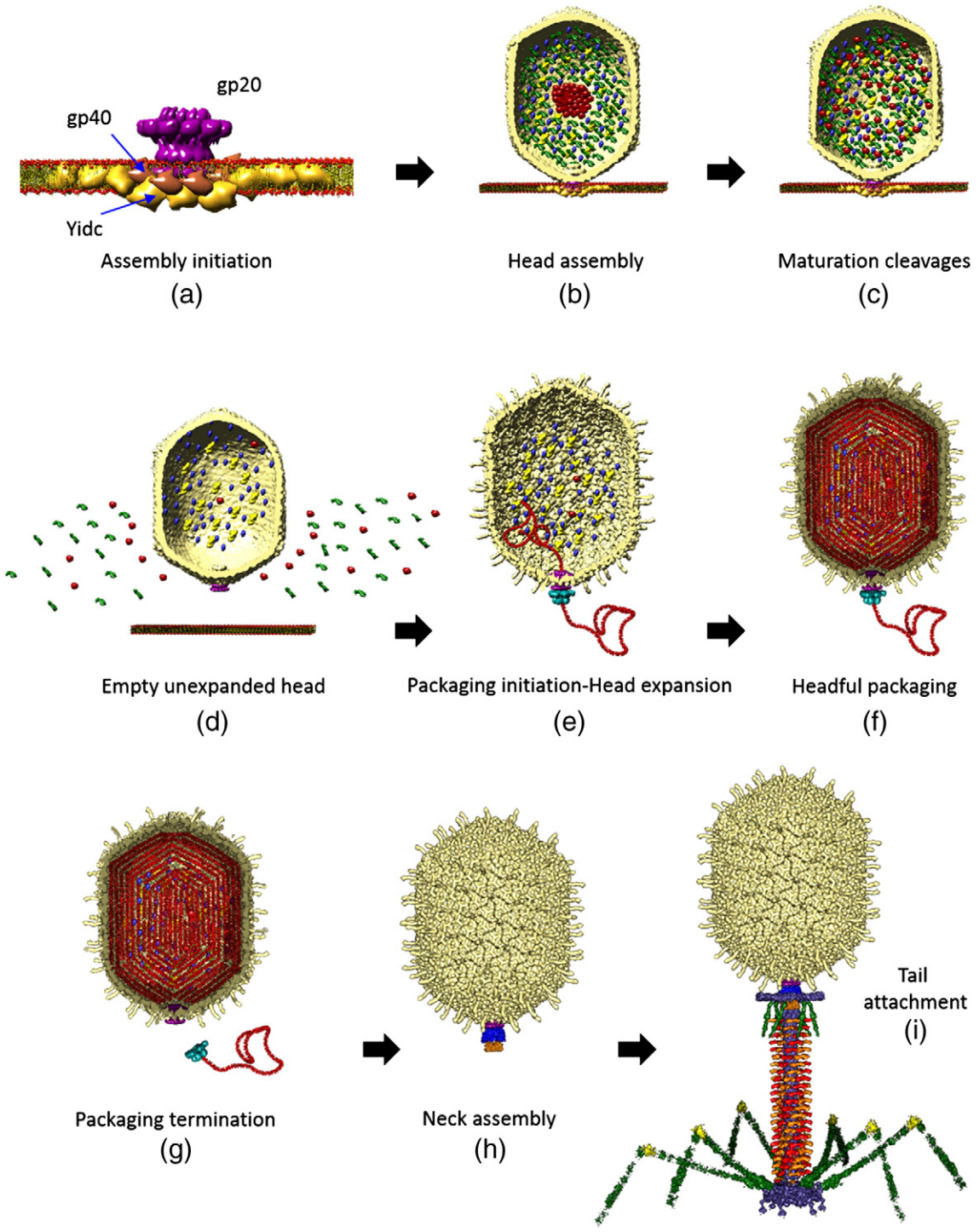

Fig. 1. Schematic of phage T4 assembly showing the functional roles of portal. (a) A dodecameric portal (magenta) is assembled on the inner membrane of *E. coli* with the assistance of the phage-coded chaperone gp40 (brown) and the *E. coli* chaperone YidC (yellow). The portal assembly acts as an initiator for head assembly, leading to co-polymerization of the major capsid protein gp23 and the scaffolding proteins gp21 (protease), gp22, gp67, gp68, IPI, IPII, IPIII, and gpAlt (b). A symmetry mismatch is created between the fivefold capsid and the dodecameric portal. Following maturation cleavages by gp21 protease (c), the cleaved prohead is released from the membrane and the scaffold proteins degraded to small peptides that diffuse out of the capsid (d). A pentameric gp17 motor assembles on the portal, and packaging is initiated. The proheads expand after about 25% of the genome is packaged (e). Packaging continues until the head is filled with the 171-kb genome (headful packaging) (f). The packaging motor dissociates (g) and neck proteins (gp13, gp14, and gp15) assemble on the portal (h). Tail and tail fibers assemble to produce an infectious virion (i).