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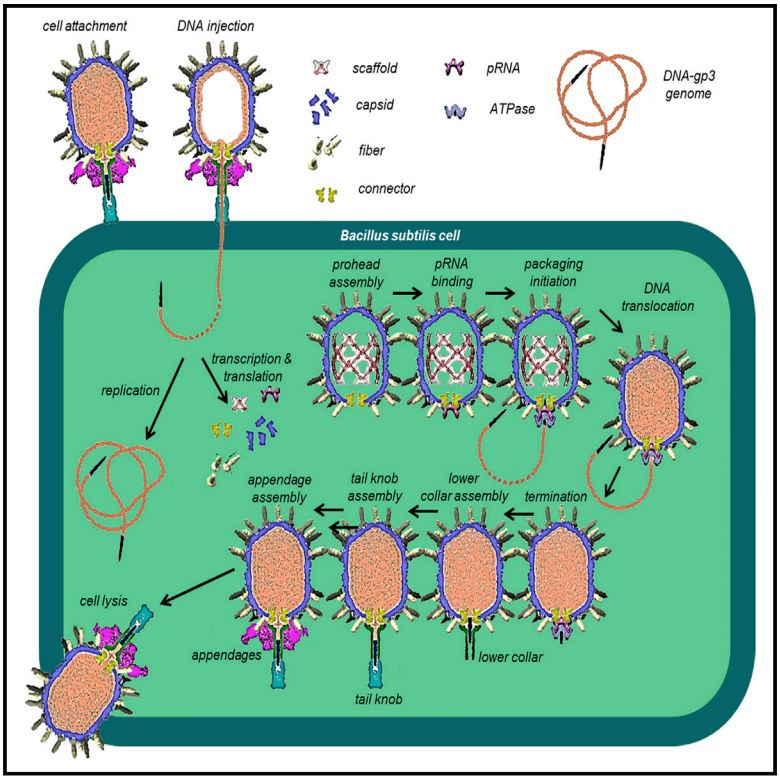
Structural and Molecular Basis for Coordination in a Viral DNA Packaging Motor

Graphical Abstract



Highlights

- A nearly complete pseudo-atomic model of a viral dsDNA packaging motor
- Two distinct motor-DNA contacts observed in an actively packaging motor
- Identified *trans*-acting arginine finger involved in inter-subunit communication
- Proposed mechanism linking ATP hydrolysis, DNA movement, and subunit coordination

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In Brief

In a remarkable process, dsDNA viruses work against entropy and electrostatic forces to package their genomes into pre-assembled protein capsids to near crystalline density. Mao et al. describe the structure of the bacteriophage phi29 DNA packaging motor and present biochemical analysis of functional elements involved in motor coordination and translocation.

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Structural and Molecular Basis for Coordination in a Viral DNA Packaging Motor

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SUMMARY

Ring NTPases are a class of ubiquitous molecular motors involved in basic biological partitioning processes. dsDNA viruses encode ring ATPases that translocate their genomes to near-crystalline densities within pre-assembled viral capsids. Here, X-ray crystallography, cryoEM, and biochemical analyses of the dsDNA packaging motor in bacteriophage phi29 show how individual subunits are arranged in a pentameric ATPase ring and suggest how their activities are coordinated to translocate dsDNA. The resulting pseudo-atomic structure of the motor and accompanying functional analyses show how ATP is bound in the ATPase active site; identify two DNA contacts, including a potential DNA translocating loop; demonstrate that a *trans*-acting arginine finger is involved in coordinating hydrolysis around the ring; and suggest a functional coupling between the arginine finger and the DNA translocating loop. The ability to visualize the motor in action illuminates how the different motor components interact with each other and with their DNA substrate.

INTRODUCTION

The ability to interconvert various forms of energy is an essential feature of living systems. Biological molecular motors accomplish this task by coupling the making and breaking of high-energy covalent bonds to conformational changes in large macromolecules. Among these, the homomeric ring NTPases are a sub-group of the large ASCE (Additional Strand Catalytic E [glutamate]) NTPase superfamily whose members are involved in numerous macromolecular force-generating tasks including chromosome segregation, DNA recombination/strand separation/conjugation, protein degradation, and the generation and maintenance of concentration gradients and electrostatic potentials (Burroughs et al., 2007; Mitchell et al., 2002; Singleton et al., 2007; Thomsen and Berger, 2008). In these motors, several energy-generating NTPase subunits are arranged as a ring, and coordinated hydrolysis of NTP molecules in the ring induces conformational changes in the motor that are coupled to the translocation of a polymeric substrate. Understanding the mechanisms by which these motors operate will illuminate the general mechanistic principles of molecular partitioning in biology as well as provide insight into the fundamental question of how chemical energy is converted to mechanical work in biological systems.

Double-stranded DNA viruses, including herpesviruses and tailed bacteriophages, encode for homomeric ASCE ring ATPases that they use to package their genomes into preformed protein shells (capsids) (Mitchell et al., 2002; Morais, 2012). The process of genome encapsidation is remarkable since considerable entropic, electrostatic, and DNA bending energies must be overcome to package DNA to near-crystalline densities within the confines of the capsid. Given the high forces involved in DNA compaction, packaging motors must work against substantially higher resisting forces than other ASCE motors. Indeed, viral DNA packaging motors are among the most powerful biological motors known, capable of generating forces greater than 60 piconewtons (Rickgauer et al., 2008; Smith et al., 2001). Thus, insights gained from the study of viral packaging motors will not only shed light on the basic mechanistic principles of a broad class of macromolecular motors, but can also illuminate how these principles have been adapted by viruses to generate and control the large molecular forces necessary for genome encapsidation.

Bacteriophage phi29 is an excellent model system for mechanistic studies of genome packaging since a highly efficient in vitro DNA packaging system has been developed, which has allowed packaging to be probed via multiple experimental approaches (Grimes et al., 2002; Guo et al., 1986; Morais, 2012). Extensive genetic, biochemical, and structural studies have shown that the motor consists of three macromolecular components (Figure 1A) (Morais, 2012; Morais et al., 2008): (1) a dodecameric connector protein (gene product 10 [gp10]) (Simpson et al., 2000), termed the portal protein in other phage systems; (2) a pentameric ring of a phage encoded structural RNA molecule (pRNA) (Cao et al., 2014; Ding et al., 2011; Guo et al., 1987; Morais et al., 2001; Simpson et al., 2000); and (3) a