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STRUCTURAL MECHANISMS OF POST-PACKAGING GENOME FLOW IN BACTERIOPHAGE T4

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Background: Genome flow is a fundamental aspect of all biological systems. In viruses, it involves movement of nucleic acid genomes into and out of a proteinaceous capsid. Viruses must recover their newly replicated genomes into a protective capsid shell (packaging) and then safely re-introduce them into a new host (ejection) to initiate infection. While the mechanisms of DNA genome packaging in large icosahedral bacteriophages (phages) and viruses have been extensively investigated, the post-packaging mechanisms involving retention, positioning, and ejection of packaged genome are poorly understood.

Aims: Using the tailed phage T4 as a model, we delineated the structural and assembly intermediates involved in transitioning a DNA-full head into an infectious virion particle, and then into a genome delivering supramolecular machine. These include intermediates of neck attachment, virion assembly, and genome release into *E. coli*.

Methods: Various intermediates produced either by mutant phage infection or recombinant protein expression have been purified and biochemically characterized. Molecular genetic approaches were used to analyze the functional significance of amino acids involved in assembly. Structures of the purified particles were determined to near atomic resolution by cryo-electron microscopy and cryo-electron tomography.

Results: Following termination of headful packaging, the pressurized T4 capsid containing tightly packed genome is sealed by the assembly of neck proteins gp13 and gp14. A dramatic conformational change in the portal dodecamer is evident, which expels the packaging motor while opening sites in portal's "clip" domain exposed outside the capsid for binding the gp13 neck protein. Unexpectedly, we discovered that a host protein Hfq, a nucleic acid binding protein, plugs the neck structure. Hfq apparently helps to further stabilize the sealed head as it awaits tail attachment.

After tail attachment, a genome end, likely the last packaged DNA, descends into the tail tube and precisely positions through interaction with an N-terminal DNA-binding motif of the tape measure protein (TMP) gp29. Six coiled-coil strands of TMP form the innermost tube of phage T4 tail, connected at the top end with DNA and at the bottom end with gp48 tube and baseplate.

When the tail sheath contracts and the baseplate transform from hexagon to star shape, TMP pilots the genome to the tip of the tail tube, poised for delivery. Then, when the baseplate plug is opened fully, TMP is expelled by DNA pressure and remodels into a transmembrane channel and guides the genome to flow smoothly through the *E. coli* membrane envelope into the cytosol.

Conclusion: Our studies describe the structural transitions of a complex and large *myophage* T4 in unprecedented detail. The mechanisms involve symmetry matches and mismatches, morphing, conformational transitions, and molecular remodeling that lead to genome retention, genome positioning, and genome release, precisely and efficiently.