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Three sequential states of spontaneous genome ejection in bacteriophage RB43 identify the baseplate hub as the DNA gatekeeper

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Phages have attracted attention in the area of phage therapy, as well as bioengineering and synthetic biology [1]. Detailed structural knowledge of phage virions becomes essential for understanding host recognition, infection mechanisms, and the design constraints of chimeric phages [2]. *Escherichia* phage T4 is one of the best-characterized viruses and has been the subject of extensive structural and functional studies [3, 4]. However, the structural determinants of genome release are still not fully understood. Furthermore, whether similar mechanism operates in other T4-like phages remains unknown. Bacteriophage RB43 is a pseudo-T-even myovirus belonging to the *Straboviridae* family, sharing moderate amino acid identity, yet a rather low sequence similarity with T4 [5]. Furthermore, no structural research has been conducted on RB43 previously.

In order to resolve its structure, we propagated RB43 virions in E. coli BL21 as host in LB medium, and collected cryo-EM images on a Titan Krios TEM at 300 kV. All image processing operations were performed with cryoSPARC based on corresponding symmetry. Proteins were predicted with AlphaFold 2 and AlphaFold 3, refined with Coot and ISOLDE, and validated with Phenix.

As a result, we resolved the cryo-EM structure of the complete RB43 virion at resolutions from 2.8 to 7.2 Å and atomic models of its major structural components, including the capsid, neck, tail, and baseplate. The overall architecture of RB43 closely matches T4, with notable exceptions. Furthermore, we identified three coexisting virion states in the purified sample: intact particles with extended tails, an intermediate state with contracted tails but DNA-filled capsids, and fully ejected particles with empty capsids. Comparison of the three states demonstrates that tail contraction alone is insufficient to trigger genome release. These findings reveal a possible genome release mechanism, which distinguishes RB43 from systems where neck proteins serve as the primary genome gate.

Источники и литература

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