

REGULATION OF LARGE TERMINASE SUBUNIT NUCLEASE ACTIVITY BY THE PORTAL PROTEIN IN NOVEL JUMBO PHAGE KLEB27-3

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The study of bacteriophages, viruses that infect bacteria, has greatly advanced molecular biology and biotechnology. Among these, jumbo phages, with genomes over 200 kilobases, stand out for their genomic complexity and partial independence from host machinery. Jumbo phages have a broad host range, infecting diverse bacterial species, including multidrug-resistant pathogens [1]. They play key roles in microbial ecosystems by regulating pathogenic populations, making them valuable for environmental and clinical applications. Given the growing threat of antibiotic resistance, jumbo phages hold promise as therapeutic agents.

Within the Podoviridae family, the jumbo phage KLEB 27-3, a double-stranded DNA (dsDNA) virus, provides an intriguing model for studying viral assembly and genome packaging [2]. A critical step in this process is the packaging of viral genomes into preformed capsids, facilitated by the terminase complex (TerC) and regulated by the portal protein. The large terminase subunit performs ATP-dependent genome translocation and cleavage, yet how the portal protein regulates the large subunit's nuclease function remains unclear.

This study investigates the portal protein of KLEB 27-3 aiming to identify amino acid residues responsible for regulating TerL nuclease activity. Using AlphaFold3 structural modeling, mutagenesis, and DNA curtains it aims to uncover the molecular mechanisms governing this regulation, offering insights into jumbo phage biology and genome packaging dynamics.

[1] Harding, K. R., Kyte, N., & Fineran, P. C. (2023). Jumbo phages. *Current Biology*, 33(14), R750–R751. <https://doi.org/10.1016/j.cub.2023.05.056>

[2] Duda, R. L. (2008). Icosahedral tailed dsDNA bacterial viruses. In *Encyclopedia of virology* (pp. 30–37). Elsevier. <https://doi.org/10.1016/B978-012374410-4.00754-8>