

A Jumbo Phage Forms a Nucleus-like Compartment to Evade Bacterial Defense Systems

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The battle for domination between bacteria and bacteriophages has resulted in innovative defense mechanisms and transformative molecules, including CRISPR/Cas9 and restriction enzymes. Bacteria deploy CRISPR proteins to combat phage infection, and phages have evolved to counter these using anti-CRISPR proteins.¹ In their recent paper,² Mendoza and colleagues note that ϕ KZ, a “jumbo phage” with >200 kb DNA, does not contain any anti-CRISPR genes, which is surprising considering their large genome. This prompted the question of how ϕ KZ evades the bacterial defense systems.

The authors demonstrated that ϕ KZ is resistant to a variety of CRISPR-Cas endonucleases and restriction endonucleases. They found that although ϕ KZ contains cut sites for these enzymes, these cut sites cannot be accessed by enzymes when inside the host *Pseudomonas aeruginosa* because the ϕ KZ DNA is segregated by a nucleus-like compartment (Figure 1). This nucleus-like compartment is an assembled protein barrier around the genome that protects the phage DNA, a finding in line with other reports that the phage DNA is surrounded by proteins in jumbo phages.³

The authors found that ϕ KZ is resistant to multiple subtypes of CRISPR-Cas enzymes (Cas3, Cas9, and Cas12) and restriction endonucleases (types I and II, HsdRMS and EcoRI, respectively). In the presence of CRISPR enzymes and single-guide RNAs targeting the ϕ KZ DNA, ϕ KZ could still infect *P. aeruginosa*, while the control phage JBD30 could not. Similarly, in the presence of restriction endonucleases HsdRMS and EcoRI, ϕ KZ was still infectious while JBD30 was not. Localization studies on CRISPR enzymes revealed that these endonucleases do not localize to the phage DNA, though phage protein ORF152 and host protein topoisomerase I do. Questions about what other phage or host proteins reside in or near this core and what factors determine the selectivity of access to this nucleus-like compartment remain.

To confirm that the phage DNA is not protected from enzymatic cleavage outside the protective core, the authors performed *in vitro* assays with extracted phage DNA. The extracted DNA was susceptible to cleavage by Cas9 and several restriction enzymes, including EcoRI. To “go undercover” to cut the DNA *in cellulo*, they rationally fused the phage protein ORF152, which resides within the core, with bacterial EcoRI to generate a fluorescently tagged EcoRI-ORF152. As visualized by fluorescence imaging, the fusion protein entered the core of ϕ KZ-infected cells, whereas EcoRI alone was occluded.

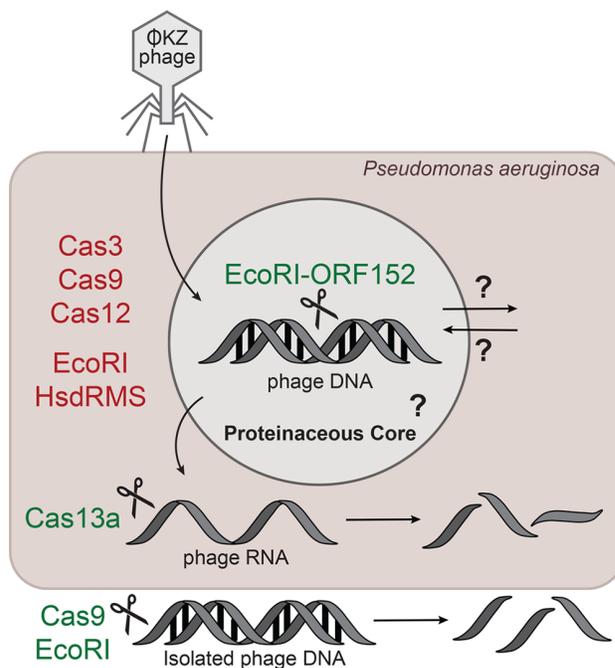


Figure 1. Upon infection of its host *P. aeruginosa*, jumbo phage ϕ KZ forms a proteinaceous core to protect its DNA from host nucleases, such as Cas enzymes and restriction endonucleases (red). However, phage RNA located outside the protein core is cleaved by Cas13a, and isolated phage DNA can be cleaved by Cas9 or EcoRI (green, below). The authors found that fusing EcoRI to ORF152, a protein that resides near phage DNA, allowed phage DNA cleavage. Some questions remain about the makeup of the proteinaceous core and what can travel into and out of the core (highlighted by question marks).

ORF152 fused to wild type EcoRI effectively reduced the phage infectivity, while phage treated with a fusion containing the catalytically dead mutant EcoRI (E111G) remained

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infectious. Because ORF152-tagged Cas9 could not enter the core, both the nature and the size of the protein determine the core accessibility. Further characterization of the core structure and mechanisms by which it permits the passage of certain macromolecules but not others should be conducted.

The phage mRNA can be transported out of the nucleus-like core to the cytoplasm for protein synthesis, providing opportunities for targeting via RNA-targeting nucleases. To overcome the naturally occurring ϕ KZ pan-resistance to DNA cleavage and boost bacterial immunity, the researchers introduced Cas13a from *Listeria seeligeri* to target different phage mRNAs in infected *P. aeruginosa*. The RNA-guided RNA nuclease Cas13a degraded the ϕ KZ mRNA by sequence-specific targeting and by indiscriminately cleaving the phage RNA (collateral damage), providing immunity against the phage. However, the existence of Cas13a escaper phages demonstrates the ability of bacteriophages and bacteria to continue to co-evolve as one develops strategies to outsmart the other.

Although the authors have made significant progress in demonstrating an essential function of this proteinaceous core, many questions remain. (1) What is the makeup of the core? Specifically, what proteins are present, and are there lipids or other molecules holding it together? How is the integrity of the shell protected against bacterial enzymes? (2) What is the makeup of these core proteins? For example, do they contain positive charges akin to histones that wrap DNA and provide further protection against endonucleases? (3) What are the other functions of this core besides DNA protection? (4) How does mRNA (or other proteins or macromolecules) leave (or enter) this nuclear core? (5) What other phages have this mechanism of pan-resistance, and is this mechanism found in all jumbo phages? It should be noted that another recent paper established that jumbo phage PCH45 has similar mechanisms of resistance to DNA-targeting Cas enzymes and is also susceptible to RNA-targeting Cas enzymes.⁴

Overall, the authors demonstrate that the protected DNA core is the mechanism of ϕ KZ pan-resistance to CRISPR and endonuclease enzymes, which is a novel defense mechanism. Considering the high entropic cost of organizing the DNA in this fashion, the authors postulate that in addition to evading host nucleases, other benefits of spatially organizing the large phage genome include replication and packaging advantages, as well as self-protection from phage nucleases, both of which remain to be addressed. Finally, the identification of such “broad-spectrum” phages that are fully equipped to fight against bacterial immunity could be promising in phage therapy against multidrug-resistant bacteria. Additionally, this study opens up new avenues to target pan-resistant phages using CRISPR systems targeting RNA, both for protecting bacteria and for effective therapeutics against viruses that infect humans.

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Notes

The authors declare no competing financial interest.

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