independent screening efforts. In order to fill in the topography of the evolving cancer dependency map, more cancer cell lines with broader genetic diversity need to be analyzed. The potential for combining larger data sets will allow us to complete the dependency map sooner and hopefully unveil a treasure trove of new therapeutic cancer targets.

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DOI: 10.1089/crispr.2020.29082.jco

A Jumbo Formation in the Viral Game Plan

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Two reports describe the surprising “pan-CRISPR” resistance of jumbo phages against DNA-targeting CRISPR-Cas systems by erecting a nucleus-like structure during infection.

More than a century after their discovery, bacteriophages (phages) are still full of unknowns. More scientists should be studying their biology, as they are a treasure trove of surprises! One source of astonishment is how they cope with the mechanisms used by bacteria to disable them. The seemingly endless arms race between phages and their hosts has led to the emergence of elaborate defense systems dedicated to the protection of the bacterial cell.¹ In parallel, phages have mounted very successful and diverse countermeasures that have helped them to become the most abundant biological entities on the planet.² These offensive and defensive schemes also became extraordinary sources of research tools in the biological sciences.

The relatively recent discovery and understanding of CRISPR-Cas systems has revealed the existence of an

adaptive immune system in bacteria and archaea, which can efficiently defeat phages. As *CRISPR Journal* readers well know, this natural defense system generally works in three steps. First, the system incorporates short “spacers” from invading nucleic acids, such as phage genomes, into the CRISPR array. These spacers, which serve as memories of past invasions, are then transcribed and processed into short CRISPR RNAs (crRNAs) that will bind to Cas proteins. Finally, the crRNAs serve as guides for Cas endonucleases to bind and cut complementary sequences during subsequent invasion of viral genomes.³

Viruses use various ways to escape CRISPR-Cas systems. For example, spontaneous mutations occurring during viral replication can prevent recognition of the targeted sequence.⁴ Another means to block the CRISPR-Cas system is to modify its nucleotides.⁵ Recently,

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studies have shown the existence of families of phage proteins that directly inhibit CRISPR-Cas systems—the so-called anti-CRISPR proteins (ACR).⁶ Each of these ACR families is specialized in the inhibition of a particular CRISPR-Cas type and/or subtype, and several modes of action have been reported.⁷

By analyzing additional phages able to infect CRISPR-immune strains, two groups have recently reported an

exciting new viral mechanism used to bypass CRISPR-Cas systems.^{8,9} Indeed, phages phiKZ and PCH45 can replicate on strains of *Pseudomonas* and *Serratia*, respectively, which, based on the CRISPR-Cas mode of action described above, they should not.

A pair of exciting reports from the labs of Joseph Bondy-Denomy and Peter Fineran solved the conundrum. It turns out that these bacterial viruses, nicknamed

FIG. 1. Nucleus-like phage shell impacts bacterial defenses. A phage proteinaceous shell protects the jumbo phage double-stranded DNA genome from degradation by CRISPR-Cas systems and restriction enzymes. However, the shell does not protect phage mRNAs from RNA-targeting CRISPR-Cas systems (types III and VI-A), as they can reach the bacterial cytoplasm. (Images are reproduced from Smart Servier Medical Art.)

“jumbo” because they have a large genome and capsid, are “resistant” to endogenous DNA-targeting CRISPR-Cas systems, such as subtypes I-C, I-E, and I-F, and to exogenous CRISPR-Cas systems from other bacterial species, such as type II-A from *Streptococcus pyogenes* or V-A from *Moraxella bovoculi*. Interestingly, these phages can also sidestep two types of restriction enzymes. The expression of HsdRM (R-M system type I) or EcoRI (type II) in *Pseudomonas* did not protect the cells against Jumbo phage infection. Bioinformatic analyses of their large genomes did not identify mutations, DNA modifications, or a known ACR, opening up the field to perhaps a new twist. And it did.

Three years ago, researchers showed that jumbo phages infecting *Pseudomonas* are able to form a proteinaceous shell structure inside a bacterial cell, reminiscent of a eukaryotic nucleus, where the phage replication and transcription take place.^{10,11} The shell is an assembly of one protein and is positioned mid-cell via the action of a tubulin-like protein, PhuZ. Homologous genes encoding proteins involved in shell assembly and localization reside in the genome of the jumbo phages used in these studies.

Using fluorescent microscopy⁹ and fluorescent confocal microscopy,⁸ the Bondy-Denomy and Fineran groups demonstrated that key intracellular proteins of the CRISPR-Cas system (as well as the restriction enzymes) are blocked outside the phage shell, preventing access to the viral genome. In fact, this viral barrier is somewhat selective, as only proteins involved in phage DNA replication and transcription can reach inside the shell. Interestingly, fusion of the EcoRI restriction enzyme with a phage recombinase allows internalization of the endonuclease and specific cleavage of the jumbo phage’s double-stranded genomic DNA (dsDNA). A similar strategy did not work with the large effector proteins/complexes of CRISPR-Cas systems. These observations support the idea that the phage shell is able to filter proteins needed inside the shell and thwarts host defenses, thereby explaining the “pan-CRISPR” resistance of jumbo phages.

Shell Game

While most CRISPR-Cas studies have been on types that target DNA, a few systems can also target RNA. Both groups exploited this CRISPR-Cas diversity. They were able to block jumbo phage replication using bacterial strains carrying RNA-targeting CRISPR-Cas systems, namely a type III system in *Serratia* and type VI (cloned from *Listeria*) in *Pseudomonas*, suggesting that the bacterial immunity was achieved by targeting phage

mRNAs. It was also the first experimental demonstration that a type VI CRISPR-Cas system can target viral transcripts of a dsDNA phage to provide protection. *In silico* analyses of bacterial genomes to search for spacers matching parts of jumbo phage genomes revealed that they were found only in CRISPR arrays associated to a type III CRISPR-Cas system, supporting the experimental data.

The protective effect of the shell against bacterial defenses in two unrelated viral isolates suggests a widespread mechanism in jumbo phages. As CRISPR-Cas and restriction-modification systems are compatible inside a bacterial cell to improve overall phage resistance,¹² the production of a shell is a crafty viral response to combat two prevalent host defenses. Yet, RNA-targeting CRISPR-Cas systems impeded the proliferation of these jumbo phages. It is tempting to speculate that some bacteria may have evolved their CRISPR-Cas systems to counteract such phage shells or perhaps they are by-products of the defense arsenal against unknown RNA phages. As the nucleus-like structure appears to require one protein, one could envision smaller phages producing a similar protective device, but the production of a shell is also likely imposing a fitness cost, thereby affecting the rate of phage replication. This burden may not be suitable for most phages.

More generally, the presence of a replication compartment for DNA viruses to enhance their replication and protect their genomes has been known for several years in eukaryotic viruses.¹³ Moreover, a recent study highlighted the existence of an anti-phage system similar to existing ones in animal cells¹⁴ and adapted phage responses likely exists as well. The presence of numerous viral genes coding for phage proteins with unknown functions is still surprising to this day. If some of them are involved in counter defenses, more trick plays are surely waiting.

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