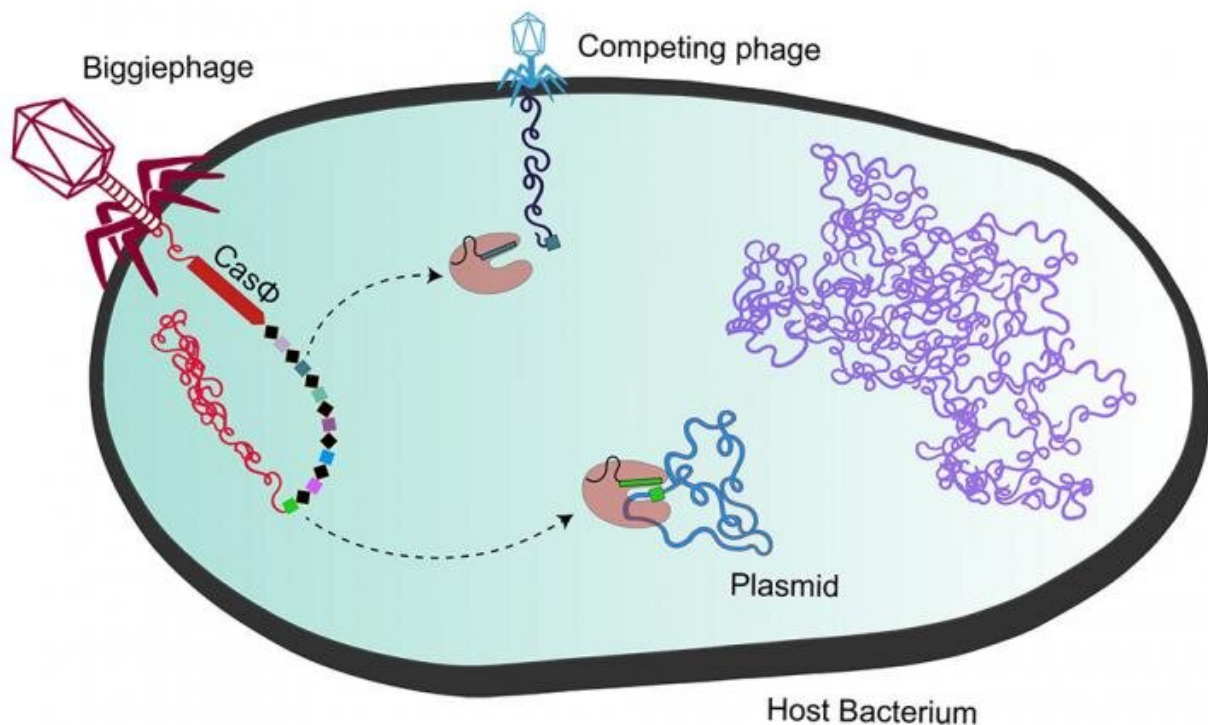


Megaphages harbor mini-Cas proteins ideal for gene editing

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A megaphage (left), a member of a bacteriophage family Biggiephage, injects its DNA -- including genes for Cas Φ (red) -- into bacterial cells to turn the bacteria against the phage's competitor (top). The reddish Pac-Man-like figures are Cas Φ proteins, enzymes that cut up viral DNA. The genome of the bacterium is shown in purple. Credit: Basem Al-Shayeb and Patrick Pausch, UC Berkeley

The DNA-cutting proteins central to CRISPR-Cas9 and related gene-editing tools originally came from bacteria, but a newfound variety of Cas proteins apparently evolved in viruses that infect bacteria.

The new Cas proteins were found in the largest known bacteria-infecting viruses, called bacteriophages, and are the most compact working Cas variants yet discovered—half the size of today's workhorse, Cas9.

Smaller and more compact Cas proteins are easier to ferry into cells to do genome editing, since they can be packed into small delivery vehicles, including one of the most popular: a deactivated [virus](#) called adeno-associated virus (AAV). Hypercompact Cas proteins also leave space inside AAV for additional cargo.

As one of the smallest Cas proteins known to date, the newly discovered Cas Φ (Cas-phi) has advantages over current genome-editing tools when they must be delivered into cells to manipulate crop genes or cure human disease.

"Adenoviruses are the perfect Trojan horse for delivering gene editors: You can easily program the viruses to reach almost any part in the body," said Patrick Pausch, a postdoctoral fellow at the University of California, Berkeley, and in UC Berkeley's Innovative Genomics Institute (IGI), a joint UC Berkeley/UCSF research group devoted to discovering and studying novel tools for gene editing in agriculture and human diseases. "But you can only pack a really small Cas9 into such a virus to deliver it. If you would have other CRISPR-Cas systems that are really compact, compared to Cas9, that gives you enough space for additional elements: different proteins fused to the Cas protein, DNA repair templates or other factors that regulate the Cas protein and control the gene editing outcome."

Apparently these "megaphages" use the Cas Φ protein—the Greek letter

Φ , or phi, is used as shorthand for bacteriophages—to trick bacteria into fighting off rival viruses, instead of itself.

"The thing that actually made me interested in studying this protein specifically is that all the known CRISPR-Cas systems were originally discovered in bacteria and Archaea to fend off viruses, but this was the only time where a completely new type of CRISPR-Cas system was first found, and so far only found, in viral genomes," said Basem Al-Shayeb, a doctoral student in the IGI. "That made us think about what could be different about this protein, and with that came a lot of interesting properties that we then found in the lab."

Among these properties: Cas Φ evolved to be streamlined, combining several functions in one protein, so that it can dispense with half the protein segments of Cas9. It is as selective in targeting specific regions of DNA as the original Cas9 enzyme from bacteria, and just as efficient, and it works in bacteria, animal and plants cells, making it a promising, broadly applicable gene editor.

"This study shows that this virus-encoded CRISPR-Cas protein is actually very good at what it does, but it is a lot smaller, about half the size of Cas9," said IGI executive director Jennifer Doudna, a UC Berkeley professor of molecular and cell biology and of chemistry and a Howard Hughes Medical Institute investigator. "That matters, because it might make it a lot easier to deliver it into cells than what we are finding with Cas9. When we think about how CRISPR will be applied in the future, that is really one of the most important bottlenecks to the field right now: delivery. We think this very tiny virus-encoded CRISPR-Cas system may be one way to break through that barrier."

Pausch and Al-Shayeb are first authors of a paper describing Cas Φ that will appear this week in the journal *Science*.

Biggiephages carry their own Cas proteins

The Cas Φ protein was first discovered last year by Al-Shayeb in the laboratory of Jill Banfield, a UC Berkeley professor of earth and planetary science and environment science, policy and management. The megaphages containing Cas Φ were part of a group they dubbed Biggiephage and were found in a variety of environments, from vernal pools and water-saturated forest floors to cow manure lagoons.

"We use metagenomic sequencing to discover the Bacteria, Archaea and viruses in many different environments and then explore their gene inventories to understand how the organisms function independently and in combination within their communities," Banfield said. "CRISPR-Cas systems on phage are a particularly interesting aspect of the interplay between viruses and their hosts."

While metagenomics allowed the researchers to isolate the gene coding for Cas Φ , its sequence told them only that it was a Cas protein in the Type V family, though evolutionarily distant from other Type V Cas proteins, such as Cas12a, CasX (Cas12e) and Cas14. They had no idea whether it was functional as an immune system against foreign DNA. The current study showed that, similar to Cas9, Cas Φ targets and cleaves foreign genomes in bacterial cells, as well as double-stranded DNA in human embryonic kidney cells and cells of the plant *Arabidopsis thaliana*. It also can target a broader range of DNA sequences than can Cas9.

The ability of Cas Φ to cut double-stranded DNA is a big plus. All other compact Cas proteins preferentially cut single-stranded DNA. So, while they may fit neatly into compact delivery systems like AAV, they are much less useful when editing DNA, which is double-stranded, inside cells.

As was the case after Cas9's gene-editing prowess was first recognized in 2012, there is a lot of room for optimizing Cas Φ for gene editing and discovering the best rules for designing guide RNAs to target specific genes, Pausch said.

More information: CRISPR-Cas Φ from huge phages is a hypercompact genome editor, *Science* (2020). DOI: 10.1126/science.abb1400

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