Phages work together to suppress CRISPR bacterial immunity

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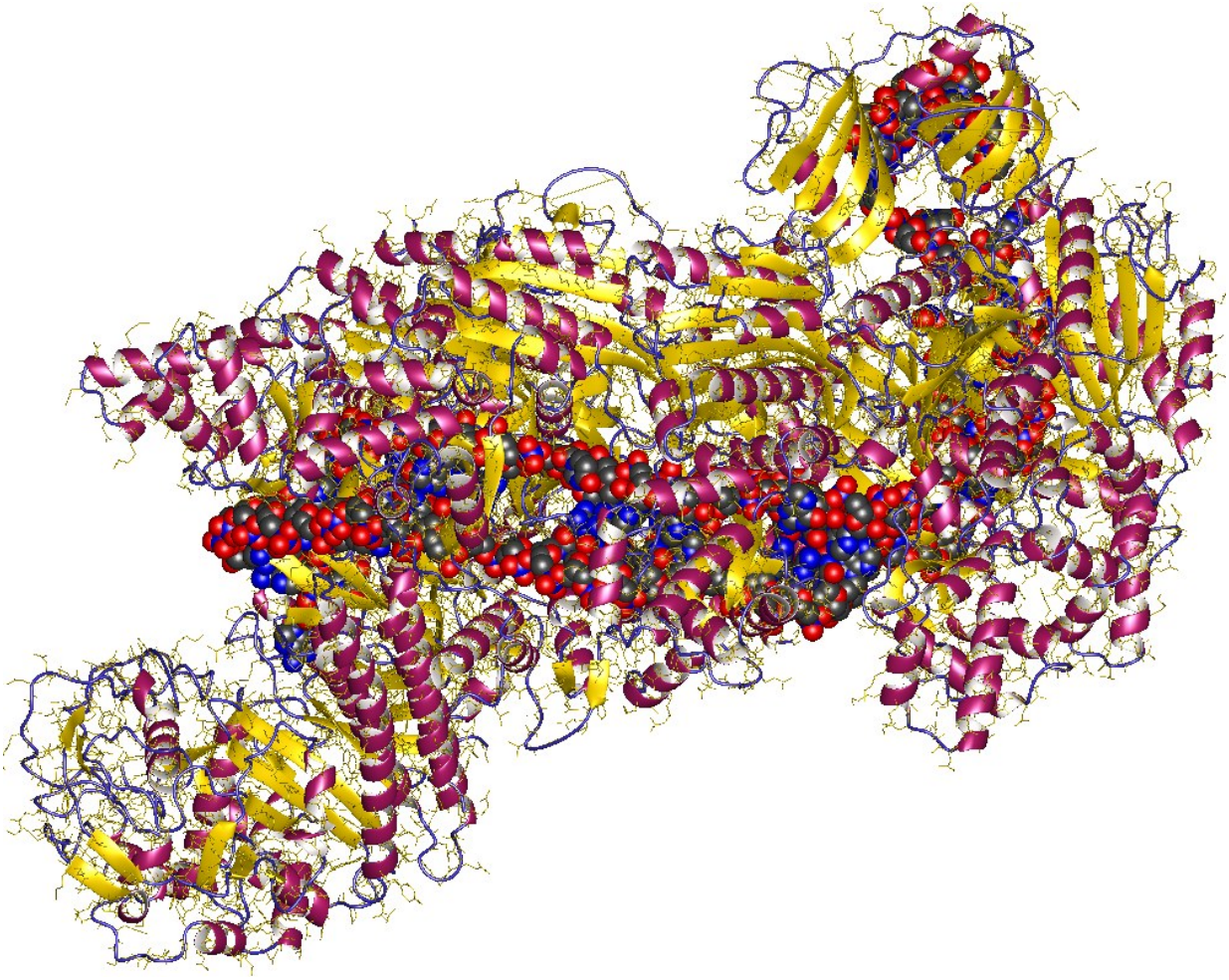

CRISPR (= Clustered Regularly Interspaced Short Palindromic Repeats) + DNA fragment, E.Coli. Credit: Mulepati, S., Bailey, S.; Astrojan/Wikipedia/ CC BY 3.0

CRISPR, or clustered regularly interspaced short palindromic repeats, are an essential part of bacterial immunity designed to defend against foreign DNA. In bacteria, CRISPR acts just like it does in human cells as a pair of scissors, in their case with the goal of cutting strands of infecting DNA. While researchers have known that CRISPR is found in roughly half of all bacteria in the wild, they did not know much about the molecular battle between CRISPRs and invading viruses or phages.

In two papers publishing simultaneously in the journal *Cell* on July 19, researchers from independent groups present evidence of phage cooperation when attacking CRISPR-containing bacteria. They discovered that to overcome destruction by CRISPR, phages have adapted by joining forces to rapidly infect a bacterium, sometimes with one phage sacrificing itself as the primer phage. Both research teams—from the University of California, San Francisco (UCSF) and the University of Exeter in England—focus on the immune relationship between bacteria and phages using CRISPR and anti-CRISPR proteins.

University of California

UCSF researchers unexpectedly discovered that phages cooperate to win the battle of time and numbers against CRISPR. To be an effective immune strategy, CRISPR-containing bacteria must quickly mount a response to phage challenge and it must do so before the phage kills the cell. "It is a pretty fast-ticking clock and a numbers game," says UCSF's Joseph Bondy-Denomy. "The CRISPR protein has to find the viral DNA very quickly, and if that doesn't happen, then the virus will proceed and kill the cell."

The team studied *Pseudomonas aeruginosa*, one of the bacteria known to pre-express a few hundred CRISPR molecules prior to infection. It can act immediately when a single phage genome enters the cell. In some other bacteria, CRISPR is initiated only when infected by a phage.

Each CRISPR protein complex contains a different guide RNA from a group of approximately 30 known guide RNAs in this type of bacteria. For the CRISPR system to be effective, it must find the target matching its guide RNA. Victory for the cell is achieved when the CRISPR machinery with the right guide RNA matches the phage that happens to be infecting at that moment, binds to the phage DNA, and cuts it. "The challenge for the phage is to produce anti-CRISPR proteins (Acr) very quickly to prevent this cutting from happening," says Bondy-Denomy, who first discovered anti-CRISPR inhibitor proteins several years ago. In this research, his team discovered that it is was impossible for a single phage genome to produce these anti-CRISPR proteins fast enough because the CRISPR proteins were already waiting.

"What we think is happening is that the first phage acts like a kamikaze phage," says Bondy-Denomy. "It gets destroyed, but along the way it starts to produce a few of these anti-CRISPR compounds that will neutralize some CRISPRs and therefore help its kin, the subsequent phage infection." His team is proposing a new model wherein the first phage contributes to the next phage's success even though that first phage is dead. Whoever wins the battle between CRISPR and phage is determined by a tipping point between the numbers and speed of CRISPR and anti-CRISPR molecule engagement. Additionally, the team discovered that not all anti-CRISPR molecules act at equivalent strengths, adding another element to the balance.

The UCSF researchers believe this phage cooperation is a form of altruism to ensure continued replication inside a host not previously reported in virus or phage models, which are generally considered as inert nucleic acids in a protein shell. They hope this finding will stimulate those working in the viral field, particularly those working on human viruses.

University of Exeter

Microbiologists at the University of Exeter also discovered that phage particles that infect *Pseudomonas aeruginosa* bacteria can work together to overcome antiviral CRISPR defenses.

The team lead by Edze Westra and Stineke Van Houte determined that some bacteria with CRISPR machinery are partially immune to anti-CRISPR-encoding phages. They demonstrated that these phages cooperate to overcome CRISPR with a first phage blocking the host CRISPR immune system, leaving behind a CRISPR-immunosuppressed bacterial host in which a second phage can successfully replicate.

Given that a single phage on its own cannot completely overwhelm CRISPR, it takes phage "teamwork" to overcome it and establish an infection in the bacterial population. As the number of CRISPR-immunosuppressed bacterial hosts in the population increase, more and more phage infections are successful, allowing the infection to spread. As a consequence, a certain number of phages are initially required to be in the environment for the phage infection to spread through the entire bacterial population. The initial amount of phages that are needed for this to occur marks a tipping point that decides whether the phage keeps replicating or whether the infection will die out.

The Exeter researchers cite this discovery as a breakthrough that may be useful for improving phage therapy, which has long been studied and tested for treating pathogenic bacterial infections. "The finding that phages act together to disarm bacterial immune systems was very surprising to us and can help to improve strategies to use phages for treating bacterial infections in humans, as the dose of phage that is used in therapy will determine if the phage can successfully eliminate the bacterial infection," says Van Houte.

"More generally, this shows that the lasting immuno-suppressive effect of a virus on its host can have profound implications for the

epidemiology of the infection," says Westra.

More information: *Cell*, Borges et al.: "Bacteriophage Cooperation Suppresses CRISPR-Cas3 and Cas9 Immunity" www.cell.com/cell/fulltext/S0092-8674(18)30738-4 DOI: 10.1016/j.cell.2018.06.013

Cell, Landsberger et al.: "Anti-CRISPR Phages Cooperate to Overcome CRISPR-Cas Immunity" www.cell.com/cell/fulltext/S0092-8674(18)30721-9 DOI: 10.1016/j.cell.2018.05.058

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