Researchers identify what flips the switch to activate a retron's toxins to prevent a viral spread

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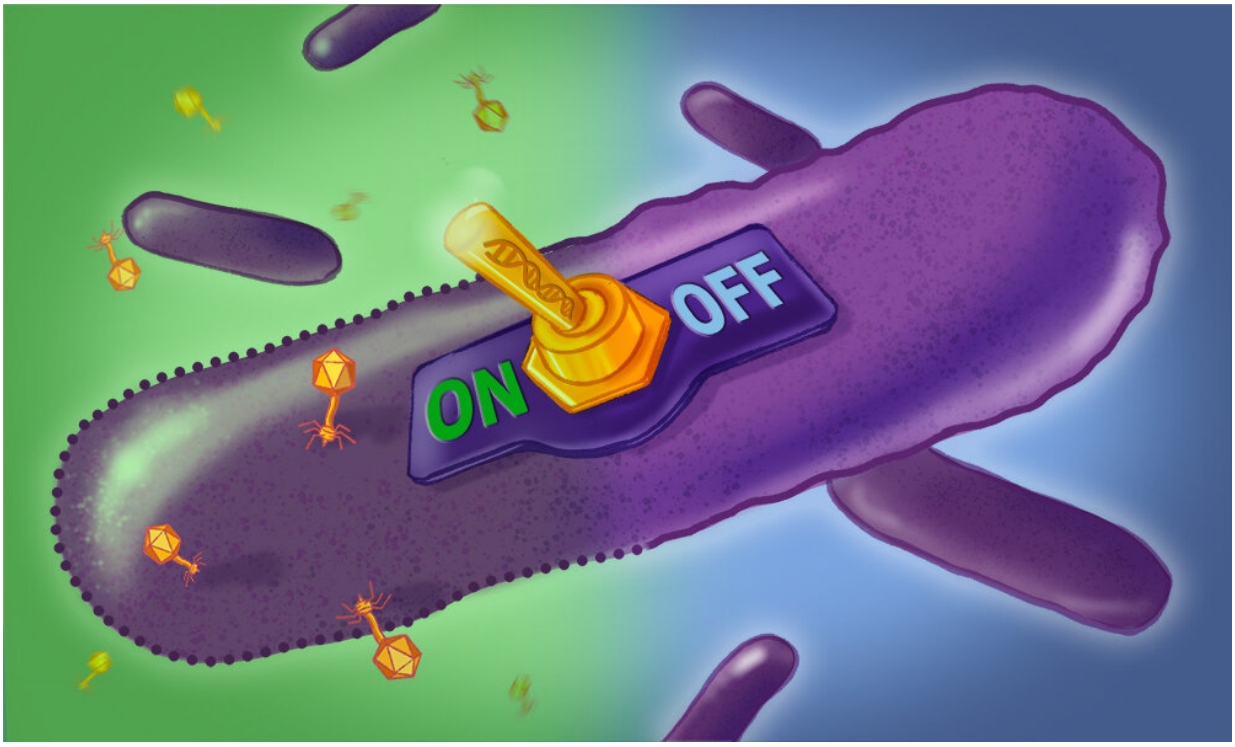

The researcher's TAC/TIC approach is able to identify what flips the switch to activate a retron's toxins to prevent a viral spread. Credit: Creative Team/EMBL

Since they were first discovered in the 1980s, retrons have puzzled researchers who simply wanted to know what these bacterial DNA

sequences actually did. Now, EMBL scientists have identified that some retrons encode toxin proteins, which they keep inactive with the help of a small DNA fragment. When a bacterial virus (phage) attacks bacteria, the small DNA can sense the attack and unleash the toxin.

"Bacterial chromosomes contain hundreds of different toxin/antitoxin systems of unknown function that might be leveraged to inhibit phages, and our findings provide an approach to understand how they could do that," said Nassos Typas, a group leader in the Genome Biology Unit and a co-chair of EMBL's Microbial Ecosystems and Infection Biology transversal themes. His group has just reported its latest findings in *Nature*.

Simply put, retrons contain an enzyme called reverse transcriptase that uses small RNA as a template to produce multicopy single-stranded DNA (msDNA). Although scientists knew how this msDNA is produced across many bacteria, its function and role in the cell had remained enigmatic until June 2020, when the Typas group, as well as the Sorek group from the Weizmann Institute of Science in Israel, posted their independent studies in an open-access preprint repository.

"For more than 30 years, we'd had no clue why bacteria have retrons because no phenotypes had been associated with cells lacking retrons or msDNA," said Jacob Bobonis, the paper's lead author, who completed his Ph.D. in the Typas group.

But new information came to light when a previous member of the Typas group found an important clue—a phenotype. They discovered that a pathogenic bacterium Salmonella cannot grow in colder temperatures without making msDNA. The group then teamed up with the lab of Helene Andrews-Polymenis at Texas A&M University and her then postdoc, Johanna Elfenbein, now PI at the University of Madison. Together, they identified that Salmonella cells unable to make msDNA

were also sensitive to a lack of oxygen, preventing them from colonizing a cow's gut.

While these phenotypes alone didn't show the retrons' special immune defense capabilities, they gave the scientists a starting point to study the retrons further.

"We quickly realized that retrons, while more complicated, looked very similar to other systems in bacteria called toxin/antitoxin systems," Bobonis explained.

Many bacteria contain hundreds of toxin/antitoxin systems in their genomes. One gene encodes a poisonous protein (toxin) that stops the growth of the bacterium, but the antidote (antitoxin) is located right next to that "poisonous" gene. While the two co-exist, bacteria grow happily. But if somehow the antidote is removed, the poison becomes active and inhibits their growth.

"Analogously, in our case, we have the retron reverse transcriptase that makes msDNA, and if we delete it, the 'toxin' is activated," Bobonis said. "We realized that the msDNA together with the reverse transcriptase form a new class of antitoxins. But we still wondered what could be that 'switch' to trigger this growth inhibition complex naturally."

These natural switches (triggers) have remained elusive for decades for all chromosomal toxin/antitoxin systems. The EMBL team decided to investigate if individual genes could act as switches. They took thousands of bacterial genes and overexpressed them one by one using robotic setups in EMBL's labs, to measure if they could trigger the toxin to inhibit the bacteria.

Ultimately, using genetics, proteomics, bioinformatics, and with the help

of other teams at EMBL (the Savitski, Zeller, and Bateman research groups), they parsed out the mechanism, discovering how viral proteins can activate as well as block these systems. They even found that retrons can thwart viral invasion on a single-cell level.

"Imagine you have 10 bacteria, and a virus goes in and infects just one of them. The virus replicates itself hundreds of times, eventually breaking the cell so that virus spills over from the infected cell, and goes on to infect the other nine cells (or more if bacteria have duplicated in the meantime). In that case, the bacterial population is killed," Bobonis explained. "In a cell where the retron is switched on by the virus, the initial infected cell withers, but so does the virus, as it needs the bacterium's machinery to replicate. Without the initially infected bacterium, the virus falters, and retrons have protected the rest of the population."

Scientists have been looking toward using phages to treat bacterial infections in humans. They call this "phage therapy," and research in this direction has dramatically increased in the past years, as antibiotics become less effective due to resistance issues. This fundamental research adds to the body of knowledge to help further that work.

An important outcome of this research is the genetics-based approach that the EMBL scientists refined: Toxin Activation/Inhibition Conjugation (TAC/TIC). Now, other scientists can also further home in on what triggers the thousands of other uncharacterized toxin/antitoxin systems. Tapping into the vast diversity of microbial functions, and uncovering new mechanisms that lie behind interactions of microbes with their environment, including their predators (phages), is at the epicenter of EMBL's new Microbial Ecosystems transversal theme.

"Since these are bacteria's internal suicide systems, knowing the trigger switches for them means that we have an angle to design artificial toxin

triggers to externally activate the toxin and kill the cell," Typas said. "Such new strategies are urgently needed as effective antibiotics become scarce to treat antimicrobial-resistant pathogens. EMBL's Infection Biology transversal theme aims to better understand antimicrobial resistance and find new ways to curtail, prevent, reverse, or bypass it."

More information: Bacterial retrons encode phage-defending tripartite toxin/1 antitoxin systems, *Nature* (2022). DOI: 10.1038/s41586-022-05091-4.

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