Model of CRISPR, phage co-evolution explains confusing experimental results

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Michael Deem . Credit: Jeff Fitlow/Rice University

A Rice University study suggests that researchers planning to use the CRISPR genome-editing system to produce designer gut bacteria may need to account for the dynamic evolution of the microbial immune system.

CRISPR is an acquired immune system that allows bacteria and other

single-celled organisms to store snippets of DNA to protect themselves from viruses called phages. The system allows a cell to "remember" and mount a defense against phages it has previously battled.

Beginning in 2012, scientists discovered they could use CRISPR proteins to precisely edit the genomes of not only bacteria but also of animals and humans. That discovery captured *Science* magazine's Breakthrough of the Year in 2015 and could eventually allow scientists to reprogram the cells of people with genetic diseases.

Despite rapid advances in the use of CRISPR for editing genomes, scientists still have many questions about how CRISPR defenses evolved in bacteria and other single-celled prokaryotic organisms. Michael Deem, a physicist and bioengineer from Rice, was first drawn to CRISPR in 2010 and has created a number of computer models to explore CRISPR's inner workings.

In a new study in the *Journal of the Royal Society Interface*, Deem and former graduate student Pu Han found there is a subtle interplay between phages and bacteria that can change, depending upon how often the two encounter one another and how quickly each evolves defenses against the other. The study documented a strange survival-extinction pattern between bacteria and phages that helps explain seemingly conflicting experimental results that have stymied CRISPR researchers.

"There's a co-evolution between the phages and the bacteria," Deem said. "The bacteria are incorporating DNA from the phages, and this allows the bacteria or their offspring to be protected against those phages.

Like all living things, phages, which attack only single-celled organisms, are constantly evolving. Deem said the rate at which they mutate and change their DNA sequence is one variable that can affect how well

CRISPR can recognize and fight them. Another factor that must be taken into account in modelling CRISPR is that the limited space available for storing phage DNA. CRISPR is constantly acquiring new snippets and throwing out old ones. An additional parameter is the encounter rate, or how often the bacteria and phages come into contact.

"If we plot the results from a simple model that incorporates these parameters, we would see that the results fall into three regions, or phases, one where CRISPR wins out and drives the phages to extinction, one where the phages win and kill off the bacteria and a third phase where the two coexist," Deem said.

Physicists often use such phase diagrams to probe the dynamics of systems. By altering the encounter and mutation rates, scientists can explore how particular combinations drive the system from one phase to another.

In the new study, Deem and Han, who is now a software engineer at Google, found that certain combinations of encounter and mutation rates produced an unexpected result, a five-region phase diagram where phages twice thrived and were twice nearly killed off, thanks to the complex interplay between the CRISPR add-drop rates and the rate at which the bacteria were exposed to phages.

"Generally speaking, we might expect that at high rates of exposure, the CRISPR immune system would drive the phages to extinction because it would encounter them often enough to have a current copy of their DNA in the CRISPR," Deem said. "In our phase diagram, we refer to this as region four, and our first interesting finding is that while extinction is likely in this case, there is always a probability, which we can calculate, that the phages will escape and not go extinct. That natural variability is of interest.

"The second point is that as we lower the exposure rate of the phages to the bacteria, and there are now fewer phages infecting the bacteria per unit of time, the bacteria have decreased opportunities to acquire DNA from the phages, and the phages can now coexist with the bacteria" he said. "We call this region three. So, we've gone from extinction to nonextinction, and we now have coexistence. That's expected and very reasonable.

"Surprisingly, we found that lowering the exposure rate even more—a case in which the bacteria now have even fewer opportunities to copy DNA into the CRISPR—resulted in another phase where the phages were driven to extinction. That's region two. And people would not have expected that."

In examining this result, Deem and Han found that the second extinction event occurred because the infection rate and the bacterial growth rate were the same, and any bacteria that acquired immunity to the phages would reproduce quickly enough to out-compete both all other bacteria and the phages. In this extinction event, a single copy of viral DNA in the CRISPR allowed the bacteria to defeat the phages. This differed from region four—the high exposure case—where many copies of DNA in the CRISPR allowed multiple strains of bacteria to defeat the phages.

Deem said the results help explain previous experimental results that have confused the CRISPR research community.

"There's been some controversy about whether CRISPR can control phages and what circumstances lead to coexistence," Deem said. "The reason for this is that various experiments have produced results from regions two, three and four. Our results clarify the range of possibilities and confirm that this range has been at least partially measured."

Deem said the findings apply only to CRISPR's use in bacterial and

prokaryotic systems. In cases where researchers are trying to use CRISPR gene-editing tools to modify those organisms or the phages that affect them, the dynamics should be taken into account.

"For example, people will eventually start editing the microbiome, the community of beneficial gut bacteria and phages that help keep people healthy," Deem said. "There's a great deal of work being done now on engineering the microbiome to make people more healthy, to control obesity or mood, for instance. For those interested in engineering the phage-microbiome interaction, it will be important to account for these co-evolutionary subtleties."

More information: Pu Han et al, Non-classical phase diagram for virus bacterial coevolution mediated by clustered regularly interspaced short palindromic repeats, *Journal of The Royal Society Interface* (2017). DOI: 10.1098/rsif.2016.0905

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