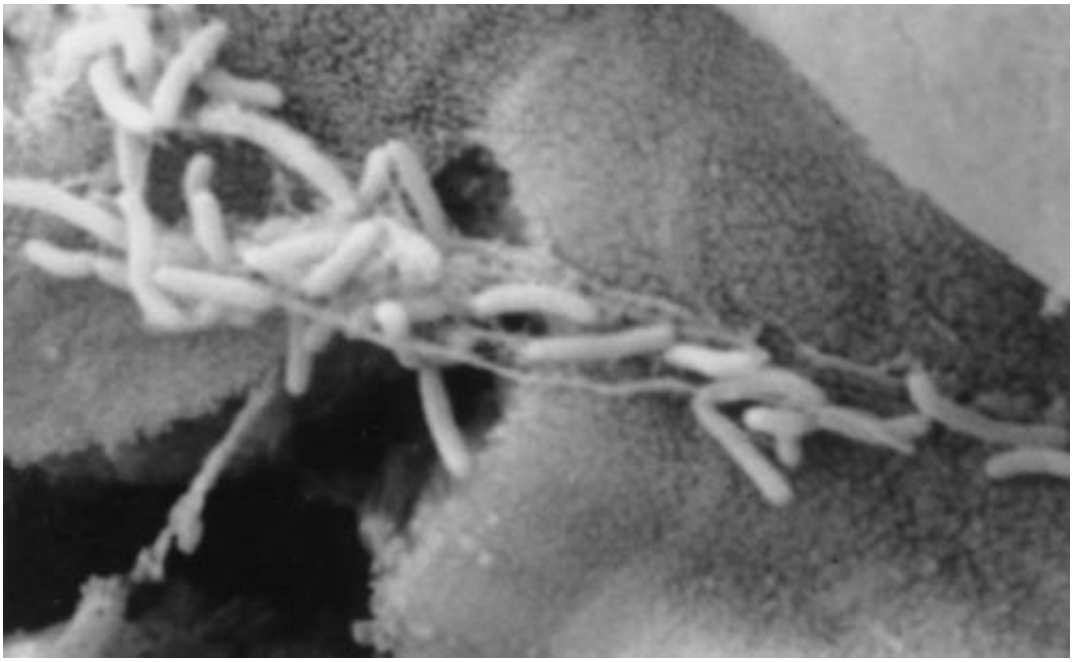
The DNA thief

May 31 2013, by David Levin



A closeup photo of Vibrio cholerae bacteria, the cholera bacteria attacked by bacteriophage viruses. Credit: Michael Angelichio and Andrew Camilli

Evolutionarily speaking, we humans are doing pretty well. Over the last few million years, we've developed big brains, social structures and more recently, culture, cities, philosophy, airplanes and the Internet. So far, so good.

Don't let these advances go to your head, though. As far as bacteria are concerned, we're nothing more than warm, squishy bags of nutrients —the perfect habitat to settle down in and make more bacteria.

This happens all the time. In some cases, our bodies coexist nicely with these tiny microbes—our intestines alone, for instance, house thousands of species that help us digest our meals. Other bacteria, like the ones that infect cuts and scrapes, however, can release dangerous toxins, and so are less welcome.

Luckily, we've evolved an immune system that can deal with unwanted infestations. As our body fights off an infection, it kills invading pathogens, and through a complex biochemical process, remembers its attackers. If the same pathogen tries to invade again, the immune system immediately recognizes and destroys it before it can spread throughout the body.

In a world where countless tiny microbes can infect and kill us, this is a handy trait to have. But as it turns out, higher life forms like humans aren't the only ones with this sort of adaptable immune response. Even some "lowly" bacteria, it seems, have a similar defense mechanism.

Like us, bacteria are in a constant battle with pathogens. In their world, bacteria-specific viruses called bacteriophages ("phages" for short) are the enemy. Phages hunt down these microbes, latch onto their cell membranes, and inject their DNA inside them. Once inside, that DNA then hijacks the bacterium's machinery and uses it to make more phages, which fill the cell from within until suddenly, like a scene from the movie Alien, they burst violently out, destroying the bacterium in the process.

Capture the flag

"I'm constantly amazed that bacteria can survive this onslaught," says Andrew Camilli. "Phages are very wily."

Camilli, a professor of molecular biology and microbiology at the

Sackler School of Graduate Biomedical Sciences, says the survival of some bacteria is partially due to a type of immune system called "CRISPR/Cas." Like a book of molecular mug shots, CRISPR/Cas lets bacteria store snapshots of DNA from viruses and other pathogens that have attacked them in the past. Using this library of usual suspects, the microbes can spot future viral infections and stop them in their tracks.

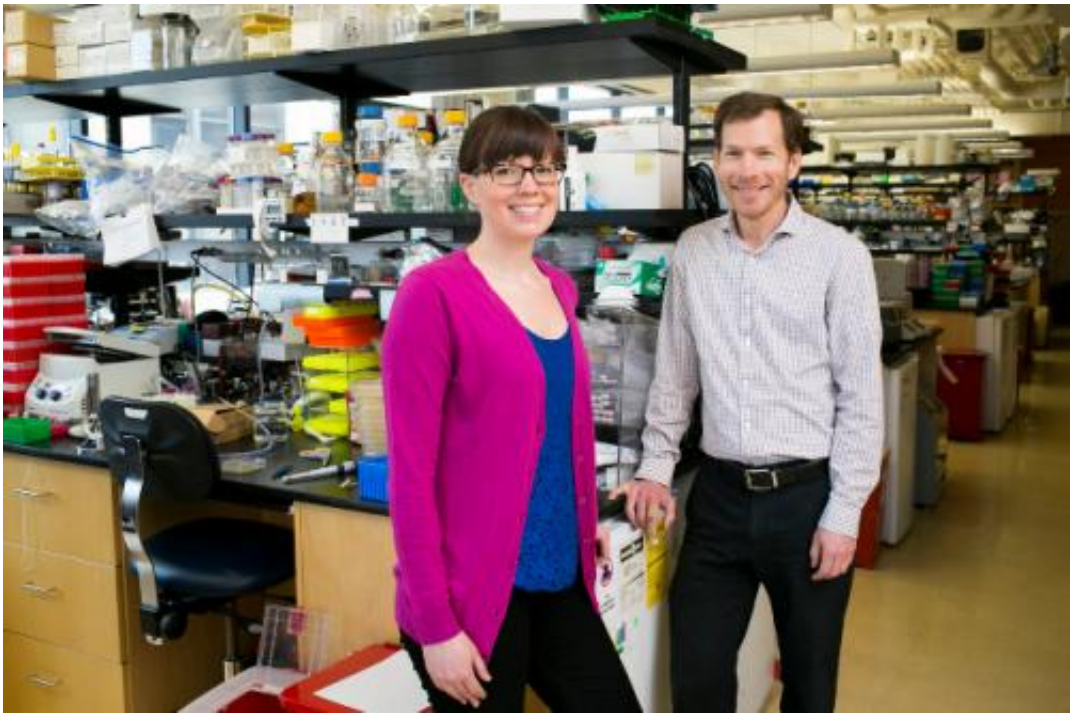
"It's really a surveillance system. It's constantly on," says Camilli, a Howard Hughes Medical Institute investigator. "If the bacterial cell is attacked by a virus that the CRISPR/Cas can recognize, it will immediately target it for destruction."

At first glance, it may seem like CRISPR/Cas, which shows up in roughly half of all known bacteria, would give the microbes the ultimate weapon in the war against viruses. Earlier this year, though, Camilli's lab showed that the evolutionary arms race between phages and bacteria has shifted in ways never before imagined. In an article published in the journal *Nature* in February, Camilli and a postdoctoral fellow in his lab, Kimberley Seed, announced that they had discovered a phage with its own CRISPR/Cas system, which it can use as a weapon to invade its bacterial hosts.

Finding a phage with an immune system like this is big news. Viruses, some researchers argue, aren't even alive. On a basic level, they're just protein shells wrapped around DNA. Yet they can evolve and change, and this latest development may indicate that they're far more adaptable than scientists had thought.

As a phage fights to invade a bacterium, its DNA can randomly intermingle with that of its host, and in the process, capture or "recombine" with bacterial genes. "Recombination of DNA happens all the time," Camilli says. "When you're dealing with billions of phages and billions of bacteria, some phage particles will often accidentally capture

some host DNA [as they invade the cell]." Once those genes are absorbed into the phages' own DNA, he notes, they remain there permanently, and are passed down from generation to generation.



Kimberley Seed and Andrew Camilli in the lab. The viruses are “just proteins and DNA—and yet they keep coming up with these amazing ways of surprising us and letting us know that they’re not so simple after all,” says Seed. Credit: Kelvin Ma

In this way, Camilli believes the newly discovered phage—called ICP1—may have stolen its entire set of working CRISPR/Cas genes from a host cell. "We now have a phage that has a weapon we didn't think a virus could use," he says. "It has captured a bacteria's adaptive immune system, and there's no example of any other pathogen—whether it be a parasite, bacteria or virus—that has done this. That's the most amazing thing of all."

A working CRISPR/Cas system, which until now had never been found in a bacteriophage, might help the virus attack and destroy a bacterium's built-in defenses, allowing it to easily invade its host, Camilli says. If that's the case, it could have big implications in medicine—once scientists understand exactly how a phage uses CRISPR/Cas to help infect and kill bacteria, it might be possible to use that knowledge to make phages that can target diseases such as cholera more effectively than existing antibiotics do.

While remarkable, this discovery isn't entirely unexpected, says Alan Davidson, a biochemist at the University of Toronto who studies phages. "It almost seemed like a matter of time until we found something the phage had done to get around bacterial defenses," he says. "With phages, it seems like almost anything that can happen will happen, eventually, because they replicate so quickly, and they're so diverse."

Stealing the code

Bacteriophages in the wild are already carrying out this sort of effective, targeted killing, says Camilli. He and Seed study phages that attack Vibrio cholerae, the bacteria responsible for cholera outbreaks in humans, and have gathered a growing body of evidence that the viruses have somehow kept the disease in check.

"It has been reported that when phage levels are really high in the environment, cholera levels are low, and outbreaks of disease are low," says Seed. "This suggests that phages play a role in controlling the bacteria population." Exactly how those phages were able to evade the bacteria's defenses, however, was a mystery, so Camilli and Seed teamed up with researchers in Bangladesh to find out. (Globally, people living in Bangladesh and India have the highest risk of contracting cholera, according to the World Health Organization.)

Together, the group analyzed frozen stool samples from cholera patients collected between 2001 and 2010, and began to catalogue the bacteriophages they found. Inside each sample, Seed says, was a different collection of phages that could attack cholera bacteria. Some of those phages came and went over the span of a decade, in some cases disappearing from the samples entirely. But slowly, Seed says, a pattern began to emerge. One single strain—ICP1—was present every time.

"We consistently saw both cholera bacteria and ICP1 in all of the stool samples," Seed says. "That told us the phage was battling the bacteria somehow, even during an infection."

To figure out how the virus worked, Seed immediately began to sequence its DNA, and after a few months of scrutiny, discovered that it held a complete set of working CRISPR/Cas genes. "I was shocked," she says. "Neither [Camilli nor I] expected to see that."

A CRISPR/Cas system might offer the ICP1 phage a distinct advantage. When ICP1 invades cholera bacteria, the system would let it split apart genes that the bacteria use to fight phage infection, rendering the cell's defenses useless. It's almost like a thief with the password to your home security system—once in the front door, he can disable your alarm before the police are alerted, giving him time to clean out your living room at his leisure.

Pathogen 1, Host 0

In an experiment, the Tufts researchers tested this notion of counter-offensive behavior on the part of the phage. They engineered a new strain of cholera bacteria—one with DNA that didn't match anything in the phages' CRISPR/Cas system. With no recognizable DNA to attack, the researchers hypothesized, the virus would be unable to destroy the bacteria's defenses. Sure enough, most mutant bacteria were able to fight

off the ICP1 phage before it could take over. But in a handful of cases, the phage won anyway—a twist that makes Camilli giddy.

"It's evidence of a race between the host and the pathogen," he says, grinning. "In those rare phages that made it, the host cell couldn't stop the phage early enough." Having won the battle, the virus could now capture bits of DNA from the bacteria, store it in its own CRISPR/Cas system, and use it against the bacteria in its next attack. Suddenly, these few lucky phages had gained a huge advantage over their fellow viruses.

It's unclear, however, exactly when or where ICP1 picked up its CRISPR/Cas system. The strain of bacteria that causes cholera in humans doesn't have the genes for CRISPR/Cas, so it seems likely that somewhere along the way, the phage must have done battle with another strain of V. cholerae that did. Regardless of where the CRISPR/Cas came from, though, Camilli believes its existence in phages could help lead to a viable alternative to antibiotics in treating cholera.

Living antibiotics

"With existing antibiotics, we're fighting a losing battle," Camilli says. "It's only a matter of time before bacteria evolve resistance to those drugs. That's the nature of the beast."

Drug-resistant bacteria are already showing up in hospitals around the world, causing life-threatening infections. With limited drug options, treatment can be difficult. Bacteriophages, however, offer a tantalizing solution. Compared to developing new antibiotic drugs, a process that takes years and millions of dollars, identifying a new phage is simple. "You can isolate a phage that can kill a specific kind of bacteria in a week," says Camilli. "Some people in the field refer to them as 'living antibiotics'—they can make more of themselves, so that's good for production—and they evolve. If the bacteria develop a resistance to a

phage, the phage can come back."

Some phage-based products are already on the market for use in the food industry. A treatment called EcoShield, for instance, has been approved by the FDA to kill E. coli bacteria on the surfaces of some foods. Although Camilli's lab showed in 2008 that similar phages could be used to prevent mice from coming down with cholera, clinical trials of phage therapies for humans are still a long way off.

"There's certainly a future for phage therapy, but I think the main roadblock is regulatory, trying to get phages certified as safe, even though these things are already living inside our bodies," says Davidson, of the University of Toronto. "Phages may not become widely used until all our existing antibiotics stop working."

The thought of a long wait for a phage-based therapy doesn't bother Camilli, though, nor does it irk Kimberley Seed. For her, the real payoff is in uncovering important details about the relationship between viruses and bacteria. "I'm a scientist. I love figuring things out," she says.

And when it comes to phages, she notes, there's a lot left to discover. "On paper, they seem very simple," she says. "They're just proteins and DNA—and yet they keep coming up with these amazing ways of surprising us and letting us know that they're not so simple after all."

More information: EcoShield

Provided by Tufts University