Turning a new phage

Matt Hansen

The idea of using bacteria-fighting viruses as a weapon against hard-to-treat infections is making a surprising comeback, but with a twist on how it has been attempted for nearly a century. Researchers and companies are now tweaking and deconstructing these bacteria killers in an effort to develop a new arsenal against antibiotic-resistant superbugs—one with more potency and a better likelihood of regulatory approval. **Lauren Gravitz** reports.

In an era when antibiotics are rapidly losing their power—when multidrug-resistant bacteria kill tens of thousands of people across Europe and the US each year—researchers have struggled to develop innovative ways to outwit the bugs' fast-evolving defenses. In May, the EU launched a public-private collaboration, called NewDrugs4BadBugs, that aims to bring together academic and industry efforts and pit them against the resistant strains. And in July, the US enacted legislation to incentivize drug companies to increase their antimicrobial pipelines.

But these efforts may not push the field as far forward as it needs to go. Most pharmaceutical companies see little financial motivation for antibiotic development, and the new government programs provide insufficient incentives for creative approaches, experts say. As a result, those drugmakers still working in this area seem to be retreading the same territory as before, relying on classic high-throughput screening methods that yield ever-smaller returns: from 1983 to 1992, 30 new antibiotics won approval from the US Food and Drug Administration (FDA); over the next ten years, that number dropped to 17. In the decade since, just seven innovative antimicrobial drugs have hit the US market.

A new approach is critically needed. Thankfully, some researchers are finding that approach by revisiting ground far older than high-throughput screens—ground so old, in fact, that it was popular before antibiotics ever existed. They are exploring and adapting an approach discovered nearly a century ago, one that employs the natural enemies of bacteria: viruses called bacteriophages.

Bacteriophages attack and reproduce by injecting their genes into their bacterial prey. The phage DNA integrates into the bacterium's own genome, where it hijacks the host's cellular machinery, allowing the virus to reproduce using borrowed resources. Eventually, the bacteria die and their cell walls burst open, freeing the phage progeny to start the cycle anew. If scientists can find a way to use this natural process to their advantage—or, better yet, engineer phages that are more effective predators—they could bolster their arsenal in the war against bacterial resistance.

The idea is gaining in popularity. "There's a renaissance going on with phages," says Timothy Lu, a synthetic biologist who studies phages at the Massachusetts Institute of Technology (MIT) in Cambridge. "And what people are doing is trying to engineer them."

Old dog, new trick

In many ways, bacteriophages are an old solution to an even older problem. They were discovered almost a century ago, first in 1915 by British microbiologist Frederick Twort and then again by the French-Canadian microbiologist Félix d'Hérelle in 1917. It was d'Hérelle who gave them the name "bacteria-

eater"—from the Greek word *phagein*, meaning to eat—and he was the first to test phage therapy, applying it against infections in livestock. He even tested its safety in humans by ingesting it himself.

In the 1910s and 1920s, d'Hérelle put the strategy to work in fighting various bacterial infections around the globe, from bubonic plague in Southeast Asia to dysentery in France. Physicians worldwide soon joined him: in India, doctors showed that swallowing cholera-specific bacteriophage eased symptoms of the disease, and in Russia they began applying topical mixtures of phage to infected lesions. Nazi war doctors in North Africa even included vials of phages in their medical kits during World War II.

D'Hérelle's most famous disciple was the Georgian doctor George Eliava. Eliava worked closely with d'Hérelle in Paris in the early 1920s and then returned to Tblisi, the Georgian capital, to establish an institute devoted exclusively to phage therapy. At its height, the Eliava Institute was producing two tons of phage product each week, primarily for the Soviet military.

The institute still exists today, with eight different research units and the most extensive phage library in the world. But in the Western world, phages fell out of favor long before the Iron Curtain came down. The advent of penicillin and related antibiotics—all of which proved easier to produce, offered a broader spectrum of action and could be taken as simple, stable pills—led scientists and clinicians in the US and Western Europe to view phage therapy as labor intensive and outdated. Bacteriophages were relegated to the laboratory and used, for example, to show that DNA, and not protein, was the carrier of genetic information.

Yet, in recent years, with antibiotic resistance on the rise and a new suite of tools in the molecular toolkit, many leading academics and industry professionals in the West have been turning back to the tiny bacteriophage for answers. Some companies are moving ahead with natural phage research; like Eliava, they are seeking out the strains best suited to attack intractable bacteria and then delivering cocktails of the bacteriophages they believe will be most effective. For example, Virginia-based AmpliPhi Biosciences has already completed a 24-person study in London involving phage therapy directed against *Pseudomonas aeruginosa* for treating adult ear infections[1], and the Swiss food giant Nestlé is testing a phage cocktail in Bangladeshi children suffering from diarrhea caused by *Escherichia coli*.

But a new cadre of scientists is taking a different tack. They are breaking the bacteriophages down and then building them back up again in the hopes of creating more deadly bacterial hit squads. These designer phages are being used as potent bactericides in the hands of some investigators, while others are working to resensitize superbugs to existing drugs that have been rendered obsolete by antibiotic resistance. "The subject of antibiotic-resistant bacteria is important enough to pursue lots of different avenues at the same time," says Mark van Raaij, a chemical biologist at the Spanish National Research Council in Madrid. "Engineering phages just seems like the most logical approach."

> "This is not phage therapy. It's a twist on phage therapy."

Improving on nature

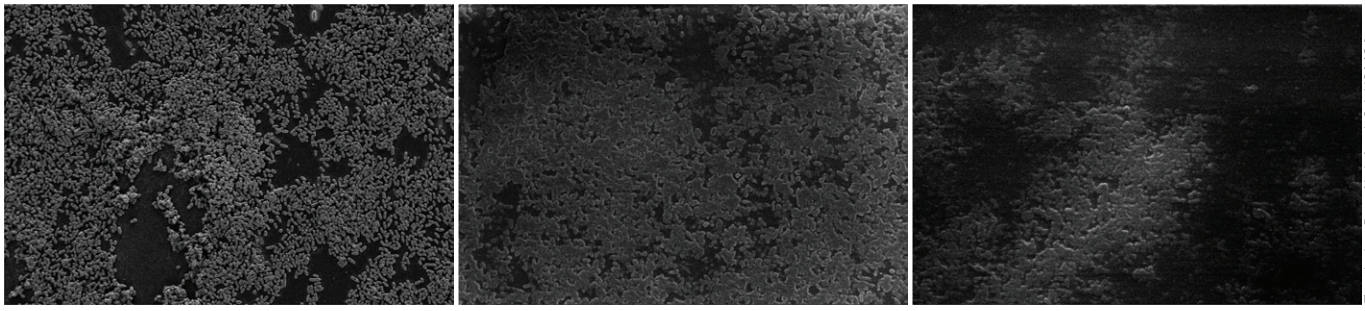
A number of obstacles have prevented natural phage therapy from finding a place in mainstream medicine. From a commercial standpoint, a long history of published research on the clinical application of phages decreases the novelty of the approach, and, thus, its patentability. "There's very little intellectual property that goes along with the phage," says Vincent Fischetti, a microbiologist at the Rockefeller University in New York.

Regulatory clearance remains another hurdle. In addition to the inherent safety concerns surrounding a live biological agent, neither the US Food and Drug Administration (FDA) nor the European Medicines Agency has an approval process in place that can easily accommodate the ever-changing combinations of phages that companies need to develop to stay one step ahead of evolving pathogens. To put it bluntly, "phage cocktails aren't compatible with how the FDA approves drugs," says Lu.

To circumnavigate these hurdles, Lu and others are modifying phages to try and create something that can be tightly controlled and more effective than natural viruses, with a potential patentability that could tempt pharmaceutical investment. In a landmark 2009 paper, Lu, together with Boston University bioengineer James Collins, took a phage that infects quinolone-resistant *Escherichia coli* and engineered it to insert a gene into the bacterium that prevents the repair of quinolone-induced chromosomal damage. Delivered in conjunction with the antibiotic, the phage increased the drug's effectiveness by up to 10,000-fold, the researchers showed[2].

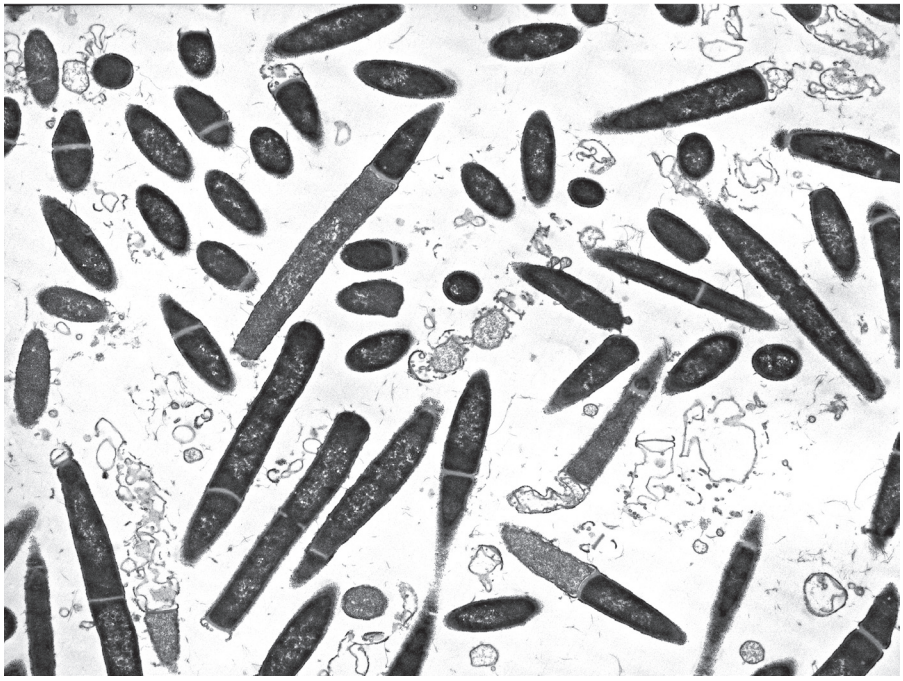
In a similar vein, Udi Qimron and his colleagues at Tel Aviv University in Israel published a study earlier this year in which, using modified phages, they caused drug-resistant *E. coli* to become susceptible again to two antibiotics: streptomycin and nalidixic acid[3]. But rather than administering the phage alongside the drugs, as Lu and Collins had done, Qimron's team applied its phage to the bugs days before the drug therapy.

In light of these findings, Qimron now hopes to develop a phage-containing spray that can be routinely applied to the surfaces of medical wards to prevent the spread of hospital-acquired infections, opening the



Timothy Lu, *Proc. Natl. Acad. Sci. USA* **104**, 11197–11202 (2007).

Breaking bad biofilms: Scanning electron micrographs of *E. coli* biofilms without treatment (left), with unmodified T7 phage (center) and with phage engineered to express an enzyme that degrades biofilms (right).



Pauline Yoong, Raymond Schuch & Vincent Fischetti

Spore war: *Bacillus cereus* bacteria after treatment with a phage lysin.

possibility for reversing drug resistance even before superbugs have infected people. "This is not phage therapy; it's a twist on phage therapy," he says. "Using this phage product every day, you will eventually replace all the resistant pathogens in a hospital with susceptible ones." Importantly, notes Qimron, because these phages aren't intended for human consumption, such a spray could be considered by regulators as an industrial product and, thus, have an easier path to FDA approval than phages formulated as ingestible or topical medicines.

In the last year, the Bill & Melinda Gates Foundation has even gotten behind phage engineering. In a recent Grand Challenges Explorations round devoted to synthetic biological solutions to global health problems, the Seattle-based organization, the largest nongovernmental funder of biomedical research in the world, awarded its first such $100,000 grants to investigate new ways of using bacteriophages for antibacterial purposes. MIT synthetic neuroscientist Feng Zhang is using his money to engineer phages capable of delivering DNA sequences encoding enzymes that prompt the bacteria to start cutting up their own genomes. Argentinean microbiologist Héctor Morbidoni of the National University of Rosario is hoping to develop a phage-based biosensor for pathogen detection. And Spain's van Raaij wants to create libraries of randomly mutated phages that can act against a wide range of different bacteria.

According to venture capitalist David Berry, a partner at Flagship Ventures, a Cambridge, Massachusetts–based firm that does not currently fund any phage-based companies, there's plenty of market opportunity to go around. Engineered phages "could very easily become a billion-plus–dollar opportunity," he says.

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Yet, despite many of the advantages of engineered phages, Graham Hatfull, cofounder of the Pittsburgh Bacteriophage Institute in Pennsylvania, foresees a future in which all sorts of phage-based approaches will be needed. "I think bacteriophages, whether natural or engineered, are likely to play a role," he says. "I like the idea of having as broad an arsenal as possible, which will include all these different types of strategies."

The sum of its parts

One of those new strategies involves extracting the bacteria-killing components of phages without having to rely on the living viruses themselves. As a graduate student in the late 1960s, Fischetti discovered that he could kill group C *Streptococcus* bacteria by applying a type of protein called a lysin isolated from phages. He purified the enzyme and found that it stripped away the mesh-like layer from the surface of the bacterial cell wall, punching holes right through the barrier and resulting in a kill that was specific and immediate[4]. Thirty years later, Fischetti returned to his doctoral work and tested the same lysin on mice with strep throat. Within two hours of oral treatment, the bacteria had completely disappeared[5]. Since then, Fischetti has uncovered phage-derived lysins that act against a range of pathogenic bacteria, including *Enterococcus faecalis*[6], methicillin-resistant *Staphylococcus aureus*[7] and even *Bacillus anthracis*[8], to name a few.

So far, Fischetti has tested his lysins only in rodent models. But through a Yonkers, New York–based startup called ContraFect, which has licensed nine of Fischetti's lysins and counts the Rockefeller scientist as one of its scientific advisors, he expects the first human trials to begin within the next year.

Meanwhile, the Indian company GangaGen Therapeutics is advancing a related approach. By combining a truncated phage lytic enzyme with part of a small molecule directed against *S. aureus*, the company has created a chimeric protein, called P128, that can kill various staph strains, including those recovered from the nostrils of human volunteers[9]. Notably, both GangaGen's and ContraFect's protein-based products will not be subject to the same difficult approval process that their parent phages would be.

Fischetti is confident that one of these many phage-based therapies will ultimately pay off. "Every two days, half the bacteria on Earth are killed by bacteriophages," he says. "It's a hugely dynamic process that's going on constantly." Thus, no matter how menacing a bacterial infection might seem, some phage somewhere has the ability to knock it down to size. Scientists now just have to find those phages—and tweak them accordingly.

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