Old dogma, new tricks—21st Century phage therapy

Karl Thiel

As antibiotic resistant bacteria threaten a public health crisis, biotechnology is turning to bacteriophages, nature's tiniest viruses. But can phage therapy overcome its historical baggage?

A US government document released in June 2000 warned of "a growing menace to all people[1]." The reference was not to terrorism or foreign dictators, but to the increasing prevalence of antibiotic-resistant microbes. Seldom given to tabloid sensationalism, an interagency task force, including the US Food and Drug Administration (FDA; Rockville, MD, USA), the US Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) and the US National Institutes of Health (NIH; Bethesda, MD, USA) concluded that "the world may soon be faced with previously treatable diseases that have again become untreatable, as in the pre-antibiotic era." Governments counsel better control of antibiotic usage in both humans and livestock, while researchers look ahead for new generations of antibiotics that can stave off new bugs. But a handful of companies are looking back into history for the answer. They are developing therapeutics based on bacteriophages, natural viruses that specifically infect—and usually destroy—bacteria (**Fig. 1**). Whether they succeed will depend on their ability to overcome the skepticism of investors, the preconceptions of the medical profession and regulatory uncertainties concerning this new class of therapeutics.

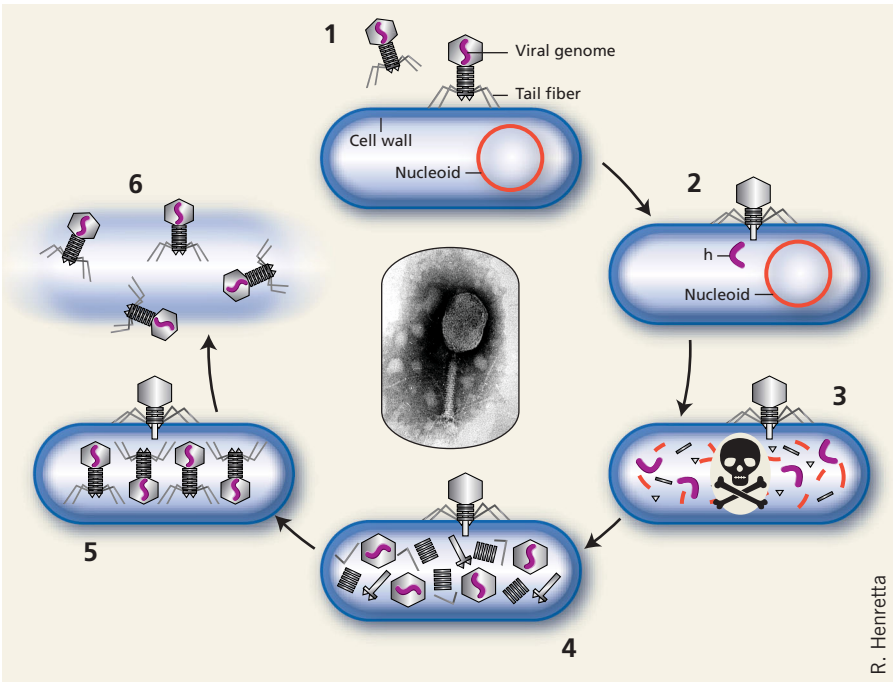

Figure 1 Phage life cycle. (**1**) Phage attaches to a specific host bacterium and (**2**) injects its DNA, (**3**) disrupting the bacterial genome and killing the bacterium, and (**4**) taking over the bacterial DNA and protein synthesis machinery to make phage parts. (**5**) The process culminates with the assembly of new phage, and (**6**) the lysis of the bacterial cell wall to release a hundred new copies of the input phage into the environment. (Graphic courtesy of GangaGen, Bangalore, India; Phage image courtesy of Elizabeth Cutter, Evergreen State University, Olympia, WA, USA)

Resistance rising

According to the CDC, methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for nearly 60% of nosocomial *S. aureus* infections in 2001—a figure that had nearly doubled over the previous decade. Although most MRSA can still be treated with powerful antibiotics, some superbugs can shrug off even the strongest drugs. The first reported case of resistance to Pfizer's (Groton, CT, USA) Zyvox, the last line of defense against MRSA, was reported a little more than a year after the drug was approved[2].

Nor is *S. aureus* the only problem. The CDC estimates that in some areas, 30% of pneumonia caused by *Streptococcus pneumoniae* is resistant to penicillin, whereas virtually all cases were susceptible in the 1970s. Vancomycin started failing to keep some *Enterococcus* (*faecium and faecalis*) infections in check in late 1988, necessitating new aggressive combination regimens. By 1993, according to the NIH, more than 10% of hospital-acquired enterococci infections reported to the CDC were attributed to vancomycin-resistant *Enterococcus faecium* (VRE). Aventis's (Strasbourg, France) drug Synercid was introduced in 1999 as a new weapon against VRE, but some resistance was observed before it even reached the market[3].

Mother Nature's little helper

A nearly forgotten therapy may yet reemerge as a savior to this accelerating crisis of

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antibiotic resistance, one that has its roots in Stalin's Russia, but which flourished briefly in the West (**Fig. 2**). For every bacterium known on this planet, there are legions of bacteriophages—tiny viruses that seek out bacteria and use them as a breeding ground, almost invariably destroying their prokaryotic host in the process. In their single-minded mission, phages ignore every cell but the strain of bacteria they have evolved to inhabit. That makes them harmless to mammalian cells and harmless even to non-target bacteria—distinguishing them from broad-spectrum antibiotics, which, when they work, can wipe out beneficial flora in the intestinal tract along with a troublesome infection. This specificity also has its drawbacks, for in order for the therapy to work, the right match between phage and bacteria needs to be determined (**Box 1**).

When a lytic phage enters a bacterium, it generally chops up the host DNA and uses it to build copies of itself. A single phage can make hundreds of progeny, which then burst out of the bacterium and go off in search of new hosts, all in the span of about 30 minutes (**Fig. 1**). Thus, a very little bit of phage can become a lot of phage in a short period of time. Bacteria, limited to ho-hum cell division, can't keep up the pace and quickly get outnumbered. Unless they can lurk in inaccessible locations, they are sought out and destroyed.

And unlike antibiotics, bacteriophages are 'living' organisms. They have been infecting bacteria since the beginning of life on this planet. Bacteria evolve to resist phage, but phage evolve, too—at an amazing rate that chemists tinkering with new generations of antibiotics can never hope to replicate. That would seem to make phages the ultimate antibacterial therapy—lethal, adaptive, highly efficient, safe to humans, all with a few billion years of experience and Mother Nature working on our side. What's not to like?

Box 1 Phage diagnostics—an early opportunity?

Since specific bacteriophages infect and destroy only a narrow range of bacterial hosts, phage therapy can be effective only if physicians know the bacterial strain they are targeting and how susceptible it is to the phage therapy of choice. When treating serious hospital-based infections, bacterial typing is a matter of course, but phage susceptibility testing is not.

But the need for phage-based diagnostics need not be another obstacle to phage therapy—it could offer an opportunity, even in guiding the use of conventional antibiotics. The process of working up a clinical specimen, identifying the bacterial species and determining antibiotic susceptibility typically takes two to three days, whereas with phage "it might be shorter," says Stanford University's Gary Schoolnik.

Indeed, in 1993 William Jacobs, a researcher at Albert Einstein College of Medicine (New York), and colleagues used phage as the core of a rapid diagnostic test for *Mycobacterium tuberculosis* (TB). A simple device called the 'Bronx Box' used inexpensive dental X-ray film to detect the glow of luciferase-engineered phage. The glow would appear only if the TB-specific phage could successfully infect its host, offering a positive identification. If that TB strain proved susceptible to a given antibiotic, the glow would be extinguished, since the reproductive source for the phage would disappear. The device offered a way of diagnosing TB without expensive X-ray equipment and shaved about a week off the time it takes for conventional susceptibility testing of this slow-growing species. Both advantages are vital to settings in developing nations where conventional lab techniques are too expensive and time consuming to be commonplace, yet dispensing the wrong antibiotics can lead to increasing resistance problems.

Carl Merril and his colleagues at the NIMH have expanded this concept. In the case of a disease like pneumonia that arises from a variety of bacterial strains, says Merril, "you can have a whole collection of phages with luciferase in them" distributed in a multiwell plate. Put a little bit of sputum in each well, and you could potentially have a result—both bacterial identification and phage susceptibility—in twenty to thirty minutes. Merril's laboratory has filed patents on this approach.

At the Rockefeller University (New York), Vincent Fischetti has developed a method of bacterial identification that works in mere seconds. This relies not on whole phage but on one of the key enzymes phages produce, lysin. Lysins, which allow phage progeny to break through a bacterial cell wall and escape, are as specific as phages themselves—each phage strain produces a lysin that works against a narrow host range. And they work almost instantly. Fischetti has demonstrated how a solution cloudy with bacteria can be cleared by the right lysin in a matter of seconds (**Fig. 3**).

Fischetti says the technique can provide a positive identification of anthrax from as few as 100 spores. And the enzyme destroys germinating anthrax within moments, without the harsh chemicals usually used for environmental cleanup. Fischetti is involved with a startup company called Enzobiotics (Columbia, MD, USA) that seeks to commercialize the technology for these and other applications. *KT*

Major obstacles

Well, to listen to critics, quite a bit. For one thing, the Eastern European example of phage therapy in practice does not offer much help to entrepreneurs hoping to navigate US and Western European regulatory agencies.

"They almost always use cocktails," says Elizabeth Kutter, a microbiologist at Evergreen State College (Olympia, WA, USA) who has observed many phage treatments in Tbilisi (Georgia). A typical off-the-shelf phage therapy for purulent infections, she says, might include 30 phage strains going after five different types of bacteria. And that preparation may vary from hospital to hospital. If that isn't enough to make heads spin at Western regulatory agencies, doctors in Eastern Europe will sometimes isolate a novel phage specifically for an individual patient with a particularly tough infection. It is a treatment paradigm that challenges the regulatory status quo in countries outside of Eastern Europe, to say the least.

Then there's the thorny issue of intellectual property (IP). Because the concept of using phages as therapeutics is almost a hundred years old, it is unpatentable. Entrepreneurs can secure protection for an individual phage strain they have characterized, but with an estimated 10^8 strains of phage in the biosphere, there's nothing to stop a would-be competitor from finding a different but perhaps equally effective strain. That sound you just heard was the wallets of venture capitalists slamming shut!

Indeed, for the handful of intrepid companies hoping to develop phage therapeutics for Western markets (**Table 1**), finding funding has been tough. Most have survived primarily on money from founders, their families and friends, or small groups of angel investors. The most significant venture capital funding in the field has gone to PhageTech, a Montreal-based company that has raised $17.1 million. But PhageTech is essentially using bacterio-

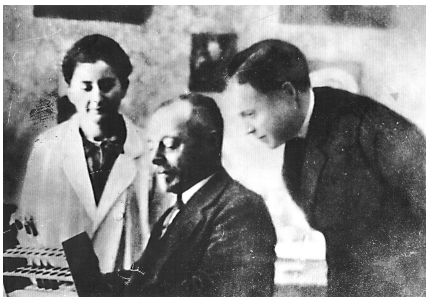

Figure 2 Phage pioneers. In 1923, French Canadian physician Felix d'Herelle, the discoverer of bacteriophage (seated) and Soviet microbiologist Giorgi Eliava (right) founded an institute dedicated to phage research, later named Eliava Institute (Tbilisi, Georgia). While initially favored by Stalin, the institute fell on hard times when Eliava was declared an 'enemy of the people' and executed. Today, people come to Eliava from around the world for phage therapy of intractable infections.

phages as scouts to find novel targets for conventional small molecule antibiotics, says CEO Pierre Etienne—an approach that professional venture capitalists are more comfortable with.

Alex Moot, a general partner at Seaflower Ventures (Boston, MA, USA) and an investor in PhageTech, says that even though his firm concentrates on the high-risk area of startup and early-stage life science companies, he avoids companies without "a proven regulatory pathway that others have established." Although PhageTech will take the tried-and-true path of small molecule drugs, that very pathway is up in the air with therapeutic phage preparations. Moot, predictably, also expressed concern about the lack of strong IP protection for phage therapeutics.

That may sound like a list of tough obstacles to overcome, but phage boosters argue that many of the problems are more about perception than reality, and that other technical challenges can be met. And there are many reasons for phage therapy to succeed despite the odds. Growing levels of antibiotic resistance and the exit of major pharmaceutical companies from antibiotic development[4] means that physicians may one day have no choice but to adopt phage therapy for a growing number of otherwise untreatable infections.

The race is on

Furthest ahead in the race to bring phage-based therapeutics to the US market is Exponential Biotherapies (Port Washington, New York, USA). Founded in 1994, Exponential is the only company among a small field of competitors to have completed a clinical trial—a phase 1 study conducted in Europe of a phage treatment for vancomycin-resistant *Enterococcus faecium* (VREF).

Exponential is planning to put this product, XpoLysin-EF, into a US phase 2 clinical trial near the end of 2004—which would mark the first FDA-sanctioned use of a phage preparation in anyone with a known infection. What the FDA will require to approve a product remains an open question, but Exponential's founder and CEO Richard Carlton has had detailed discussions with regulators on the subject.

Early indications are that the agency is proceeding with customary caution. Carlton says he first approached the FDA about using a panel of several phage as a means to broaden coverage against VREF. "They said we had to start with just a single phage strain," says Carlton.

Carlton still believes the agency may ultimately bless clinical trials and products involving phage cocktails as long as the phages are not too divergent. To get to the next stage, however, he had to go back and find a single phage strain he thought could succeed in a clinical trial. Having originally isolated a phage active against about 60% of VREF strains, researchers at Exponential were able to find one with 95% coverage, he says.

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But Carlton and most others working in the field still believe that using multiple-phage preparations will make phage therapy more effective, more practical and easier to develop. The FDA has traditionally frowned on drug cocktails unless all active components are proven safe and effective both individually and collectively. But Ryland Young, a phage biologist at Texas A&M University (College Station, TX, USA) and the executive director of research at GangaGen (Bangalore, India), asserts that such a stance is based on a tradition that has no relevance to phage therapy. "We're not talking about having two antibiotics that work by completely different mechanisms. All phages work the same way," he says. He thinks the agency will come around, but acknowledges that their failure to accept cocktails would "greatly hamstring bacteriophage therapy."

Not phage away

Not all challenges to phage therapy can be reduced to regulatory tradition and historical bias. One part of Exponential's core IP is based on another potential problem with phages. Most are cleared very rapidly by the reticuloendothelial system if they don't quickly find a bacterial host in which to multiply, meaning that an effective phage, particularly one administered systemically, could potentially be cleared from the body before it ever reaches its target. Carlton, along with National Institute of Mental Health (NIHM, Bethesda, MD, USA) senior investigator Carl Merril and others, developed a means of selecting phage that are good at evading the reticuloendothelial system through a technique called 'serial passage'—essentially passing a phage of interest through a living organism, selecting those able to get past the body's filters and repeating the process until a 'long-circulating' phage variant is isolated. If such a technique proves necessary to create an effective phage therapy, Exponential may have also effectively blocked a lot of competition, because the company holds a patent on this technique.

Carlton's competitors don't seem worried, however. At Intralytix, a phage company in Gaithersburg, Maryland, Alexander Sulakvelidze, former Director of Molecular Microbiology for the Georgian National Center for Disease Control, (Tbilisi, Georgia) believes rigorous scientific standards can establish the safety and efficacy of phage therapy resembling what is practiced in his native Georgia. Like most researchers who have reviewed the Eastern European literature on phage therapy and seen it practiced, he believes the approach works but acknowledges that the evidence, while copious, is largely empirical.

However, Sulakvelidze does not believe that it will be necessary to engineer phages in order to make them acceptable to Western regulators. "The only reason to do it is for patent protection purposes," he says. Sulakvelidze, who has been involved in one informal discussion with FDA on develop-

ing phage therapies, echoes Carlton in saying that the agency does not seem to have any fundamental problems with the idea of phage therapy, but has not decided exactly how to regulate products. "It's a learning curve for them and for us. There is no existing guideline that they can go by."

Another way

If the FDA will not accept multiple-phage cocktails without putting each component through its own clinical trial, technology may have an answer. "What's really needed here is to broaden the host range," says NIMH's Merril. "There are more than 20 common strains of *Streptococcus pneumoniae* that cause pneumonia, so if you wanted to have a universal treatment for pneumonia, you'd have to patent all those different phage and take them all through FDA. Wouldn't it be nicer if you could make a phage that would infect all 20?"

Some phage enter their chosen host via a specific enzyme that chops a hole in the capsid coat of bacteria. But Dean Scholl, a researcher in Merril's laboratory, discovered a phage that carried more than one such enzyme in its tail and could thus infect bacterial strains with two different outer coats[5]. That has led the group to explore engineering the phage further along these lines. "What stops you from doing three different enzymes?" asks Merril. "Now you end up with a phage with a much broader host range." If the FDA proves unwilling to allow researchers to use phage cocktails, such an approach may prove necessary.

Specificity and half-life aren't the only potential problems. Because phages multiply exponentially, 100-fold or more every generation, dose cannot be controlled in a customary manner. Carlton doesn't see that as a problem—he intends to track the pharmacokinetics of phage just as a clinician would any small molecule. It's just that the charts will look a little odd, with the concentration of phage surging during a lytic cycle, diminishing as the bacterial hosts are killed off and some phage are cleared by the reticuloendothelial system, then surging and diminishing again in subsequent cycles, and finally disappearing when there are no further bacteria to support propagation and the remaining phage are cleared from the body.

Lysis to kill

Still, the potential problems of an exponentially replicating therapeutic have led some researchers to wonder if phage therapy would be less complicated if cell lysis weren't part of the equation. At GangaGen, one of the newer companies to enter the field of phage therapy, a research team of 25 people in Bangalore, India, is exploring phages engineered to be endolysin deficient.

Most phages reproduce upon entering a cell, and in that process kill their host. They encode two families of proteins, holins and lysins, that allow the phage progeny to burst through the bacterial cell wall and go off in search of new hosts. But the cell is already

Box 2 Low hanging fruit—agricultural applications

Getting a phage-based human therapeutic to market means navigating some untested waters at FDA, but an easier commercial path exists for phage products. Bacterial contamination of foodstuffs is a multi-billion dollar problem affecting an estimated 76 million people annually in the US alone, according to the CDC. Bacteriophages could form the basis of products used to decontaminate food-processing plants, livestock and even farmers' fields. Getting such products to market generally means navigating the US Department of Agriculture (USDA; Washington, DC, USA) or the Environmental Protection Agency (EPA; Washington, DC, USA), which have lower regulatory hurdles to product approval. The FDA would not regulate such phage products unless they are used directly on foodstuffs.

Several phage companies are hoping some of these agricultural and environmental applications will turn out to be low-hanging fruit that will create a revenue stream to fund research into human therapeutics. At GangaGen, Janakiraman Ramachandran says his company hopes to have its first product, a phage that kills *Escherichia coli* 0157:H7 in manure, on the market in about 18 months. Manure, used as fertilizer, can contaminate groundwater if the manure comes from infected cattle. In May 2000, for instance, over 2,000 people in the farming community of Walkerton, Ontario (Canada) were made ill and seven died as a result of *E. coli* infection ultimately linked to a contaminated well.

Manure used as fertilizer is typically collected and liquefied before use; GangaGen is hoping to include an extra step in which phages that kill *E. coli* are added to the mix. The exponential reproduction of phage particles will make it possible to treat large vats of manure with relatively modest amounts of phage. The company has a subsidiary in Ottawa, Ontario, dedicated to agricultural and environmental phage R&D.

Biophage Pharma (Montreal, Quebec, Canada) is likewise working on phage products to combat *E. coli* as well as *Salmonella typhimurium*, says CEO Elie Farah. The company intends its products to be used in live animals and, in the case of *E. coli*, on carcasses before meat processing. That's particularly important for the processing of ground beef, since meat trimmings from different carcasses are combined as beef is ground, and *E. coli* from one contaminated carcass can be spread to a large amount of meat.

Intralytix, meanwhile, is concentrating on products that could be used to decontaminate food-processing facilities. In June 2002, the company received an experimental use permit from the EPA to test a phage preparation called LMP-102, active against *Listeria monocytogenes*, on nonfood contact surfaces. There are about 2,500 cases of listeriosis in the United States each year, 20% of which are fatal. Because of the seriousness of the illness, the USDA has a strict zero-tolerance policy on the bacterium in ready-to-eat foods; the detection of *L. monocytogenes* in deli meat in October 2002 was responsible for the biggest meat recall in US history.

LMP-102 has been used successfully in pilot tests in a few facilities, according to Alexander Sulakvelidze. The company, however, does not have a firm time line for commercialization. Intralytix has also asked FDA for permission to experimentally use the same preparation directly on foodstuffs, he adds. Even Exponential Biotherapies, furthest ahead in developing human therapeutics, has a food safety R&D program, with a particular focus on *L. monocytogenes* and *Campylobacter jejuni*, a microbe commonly found in chicken. Though not considered deadly, *C. jejuni* is the leading cause of bacterial diarrhea in the United States.

"One advantage of this approach is that the same phage can be used in cattle, swine, manure and even humans," says GangaGen's David Martin. "The issue is formulation and dose, but the active agent is the same." *KT*

dead by the time that happens—in terms of lethality, the lysis is just a matter of burning down the house for good measure.

"We realized that this last function is not essential for containing infectivity," says GangaGen founder and CEO Janakiraman Ramachandran. Researchers at the company replaced the lysin gene in the T4 coliphage with a green fluorescent protein gene, and determined that the engineered strain killed *Escherichia coli* but did not release progeny from the cells.

Ramachandran argues that this approach offers a number of advantages. For one thing, there is no sudden release of endotoxin that could result from the rapid lysis of bacteria and potentially become a deadly complication. In addition, it is easier to control dose. "I suspect that the regulatory agencies are going to be much more comfortable with a linearly dosed therapeutic than with one that you can't control because it exponentially replicates," says David Martin, chairman of GangaGen's board of directors.

But Evergreen's Kutter argues that the exponential replication of phage allows them to pierce progressively deeper into tissue to root out deep-seated infections. She also suggests that lysin-deficient phages "might" be attractive in some circumstances, such as infections of the gut, but points out that such phages will be in a race for survival against the reticuloendothelial system, whereas natural, replication-competent phages can mount an effective attack against a localized area of infection once they get a foothold, even if most of the initial dose is cleared away.

On the other hand, Martin notes that in many infections, an initial dose should be adequate and that with a wild-type phage, "you've generated a huge excess of phage from the last lysis," which could theoretically prove immunogenic and which a patient would potentially be "dumping back into the environment."

This last issue springs from the fact that phages actively swap around DNA among bacteria. Although most phage reproduce directly upon entering a bacterial cell, some (temperate phage) become dormant, either integrating into the host chromosome or simply living in plasmids within the cell, going into a destructive lytic cycle later. When the phage become active again, they can shuffle genes around through a process called specialized transduction. Even lytic

Table 1 Select therapeutic phage companies

Company (Location)	Focus	Web	Founded
Biophage Pharma (Montreal, Canada)	Cancer, infection/inflammation and immune modulation, *E. coli* as well as *Salmonella typhimurium* in livestock and carcasses	http://www.biophage.com/	1995
Enzobiotics/New Horizons Diagnostics (Columbia, MD, USA)	Lysin enzymes derived from phage as diagnostic agents and topical therapeutics	http://www.nhdiag.com/	2003
Exponential Biotherapies (Port Washington, NY, USA)	Phase 1 clinical trial completed of phage against VRE bacteria; also MRSA phage and biodefense	http://www.expobio.com/	1994
GangaGen (Bangalore, India; San Francisco, CA, USA; Ottawa, Canada)	Nontemperate, nonlytic phage for human therapeutic use; agricultural/environmental applications	http://www.gangagen.com/	2000
Hexal Gentech (Holzkirschen, Germany)	Phages as packages to deliver lethal plasmids to target bacteria	http://www.hexal-gentech.com/	1998
Intralytix (Baltimore, MD, USA)	Phages for environmental, food processing and medical applications	http://www.intralytix.com/	1998
MicroStealth Technologies (Cambridge, MA, USA)	Phages as delivery vehicles for antimicrobial peptides	N/A	2002
Phage Biotech (Tel Aviv, Israel)	Lytic bacteriophage technology for clinical, veterinary, agricultural, industrial and ecological applications	http://www.phage-biotech.com/	2001
Phage Therapeutic (Bothell, WA, USA)	Phages against drug-resistant human bacterial infection, now essentially defunct	N/A	1997
PhageGen (Las Vegas, NV, USA; previously Regma Bio Technologies) of London)	Therapeutic phages and lethal protein delivery	http://www.phagegen.com/	2000
PhageTech (Montreal, Canada)	Phage genomics to discover new targets for small molecule antibiotics	http://www.phagetech.com/	1997
Phage-Therapy (Tbilisi, Georgia)	Provides logistical support for patients seeking phage therapy treatment in the Republic of Georgia	http://www.phage-therapy.com/	2002
Phico Therapeutics. (Cambridge, UK)	Phages to deliver lethal proteins	http://www.phicotherapeutics.co.uk/	2000

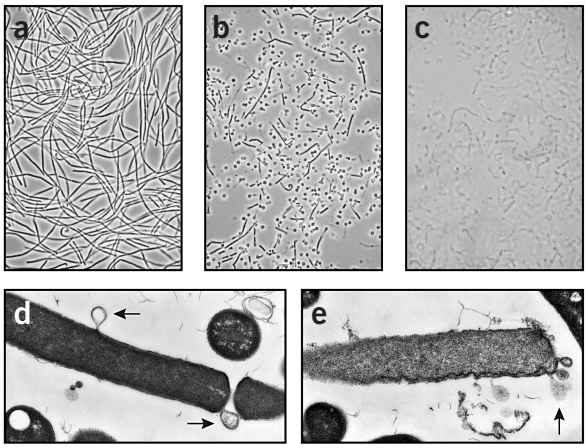

Figure 3 Lysis to kill. (**a–c**) Electron micrographs show phage enzyme wiping out a colony of *Bacillus cereus*: a healthy colony of bacteria (**a**); the bacteria 1 minute after enzyme treatment (**b**); and bacteria 15 minutes after treatment (**c**). (**d**) Lysin punches holes in bacterial outer cell wall, causing it to explode. (**e**) A single bacterium begins to rupture, its inner membrane spilling through an enzyme-induced hole. (Image courtesy of Vincent Fischetti, Rockefeller University, New York, NY.)

phage can be mispackaged with bacterial DNA through a similar process called generalized transduction. Phage can carry out random pieces of the bacterial genome, potentially including resistance or pathogenic toxin genes, and introduce them to other bacteria. Ultimately, some phage would end up in the sewer where they could theoretically introduce toxin genes to otherwise benign bacteria in the environment.

"You could argue that those are bad genes with which you are populating the environment," says Young. Preventing phage from lysing out of cells could limit the problem, since dead bacteria trapping the phage should eventually be chewed up by macrophages, but nothing is likely to eliminate the problem altogether.

What regulators will construe as safe remains for now another unanswered question. Young, for one, is quick to note that DNA transduction is going on all over the environment anyway—at any moment on planet Earth there are literally tons of DNA being swapped around by phages. "Unless you're completely compulsive, it doesn't make a whole lot of sense to me to worry about transduction," he says. Nevertheless, GangaGen and all its competitors are seeking out phages that are lytic, not lysogenic (integrate their genetic material into bacteria), and rarely engage in generalized transduction.

Phage zombies

A conceptually related approach is being pursued by a research team at the Medical University of South Carolina (Charleston, SC, USA), working in conjunction with Hexal Gentech, the research and development arm of German pharmaceutical company Hexal (Holzkirchen).

Working with the filamentous coliphage M13, the researchers created a sort of zombie phage—a regular phage body that still seeks out a specific microbial host (in this case, *E. coli*) but that has had its head emptied of the usual DNA necessary for replication. It instead injects only a lethal protein system, killing the host cell but not leading to the lysis of the cell or new phage production.

Michael Schmidt, a professor at the university and member of the research team, theorizes that the potential for bacterial resistance could be reduced by introducing multiple killing systems into the engineered phage, making it harder for the bacteria to mutate around their demise. And unlike other phage, filamentous phage do not have a strict limit on the amount of genetic information they can encapsulate, which has endeared them to many genetic engineers over the years.

The potential challenge is that filamentous phage are thought to infect only bacteria with a pilus, a spear-like protein tube required for the phage to gain entry. Not all bacterial species, nor all strains within common species, have a pilus—meaning that filamentous phage may not always be the best choice of vector for antimicrobial agents.

Nevertheless, says Schmidt, "we anticipate that with appropriate engineering, we should be able to extend the host range of our system." In any case, the researchers have also done work with the lethal protein system in P1, a nonfilamentous coliphage, and Schmidt believes the system can be adapted to other kinds of phage.

Waiting for the rules

The FDA will ultimately set the hurdles that companies must jump to put therapeutics on the US market, but for now those hurdles remain mostly undetermined. Indeed, some phage companies plan to do an end run around some regulatory obstacles by seeking first to commercialize phage products for agricultural applications, where regulations are less stringent (see **Box 2**).

GangaGen's Young, for one, thinks that companies in the field should take a proactive approach in determining the standards that phage therapies must satisfy. The first step toward that goal may be a 'Phage Summit' that Young is helping to organize, scheduled to take place next August in Key Biscayne, Florida, USA.

"I'm hoping we can pound out some common standards," he says. In the absence of a unified and scientifically grounded front among the companies, the FDA may tend toward its traditional standards, which could prove difficult for the nascent industry.

But even without success in the major Western markets, phage therapy could still score a huge victory. Gary Schoolnik, professor of Medicine, Microbiology and Immunology at Stanford University (Stanford, CA, USA) and chair of GangaGen's scientific advisory board, notes that one further advantage of phages is that they can be produced very cheaply. That could make them ideal for treating infections in developing countries—particularly enteric infections like cholera, where Schoolnik notes a preparation could be "comparatively crude and still be effective." Indeed, the very obstacles to commercializing phage in developed markets, like patentability, could actually be an advantage to developing nations that want to gain a little know-how and go it on their own.

And problems in developing countries could spread to the developed world if phage therapy fails. "I don't want my grandchildren to live in a world that is effectively in a pre-antibiotic era," says Kutter.

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