

The other microbiome

The viruses that colonize our bodies play an underappreciated role in health and disease.

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As recently as 2010, Forest Rohwer could be found immersed—literally—in the Pacific Ocean. Rohwer, a microbiologist at San Diego State University, has spent more than a decade researching the bacteria and viruses that inhabit coral reefs, developing ways to study the microbes, and asking how they interact with each other.

Now he's diving into a new territory that is prime for exploration: the viruses that call the human body home. His move comes

at a time when there is growing interest in studying the so-called human virome, and the methods that Rohwer helped develop and fine-tune for studying marine microbes are now being applied to the human ecosystem. After all, the human gut isn't all that different from a deep-sea community; it's full of viruses and bacteria struggling to survive in a tough environment.

"Almost all of the techniques that are now used to study the human virome were

developed for marine biology," says Rohwer. Just as Rohwer used high-throughput genetic sequencing to describe coral reef microbe communities, he and others are now amassing data about which viruses live in the human body, how they differ between sick and healthy people, and how the virome changes over time.

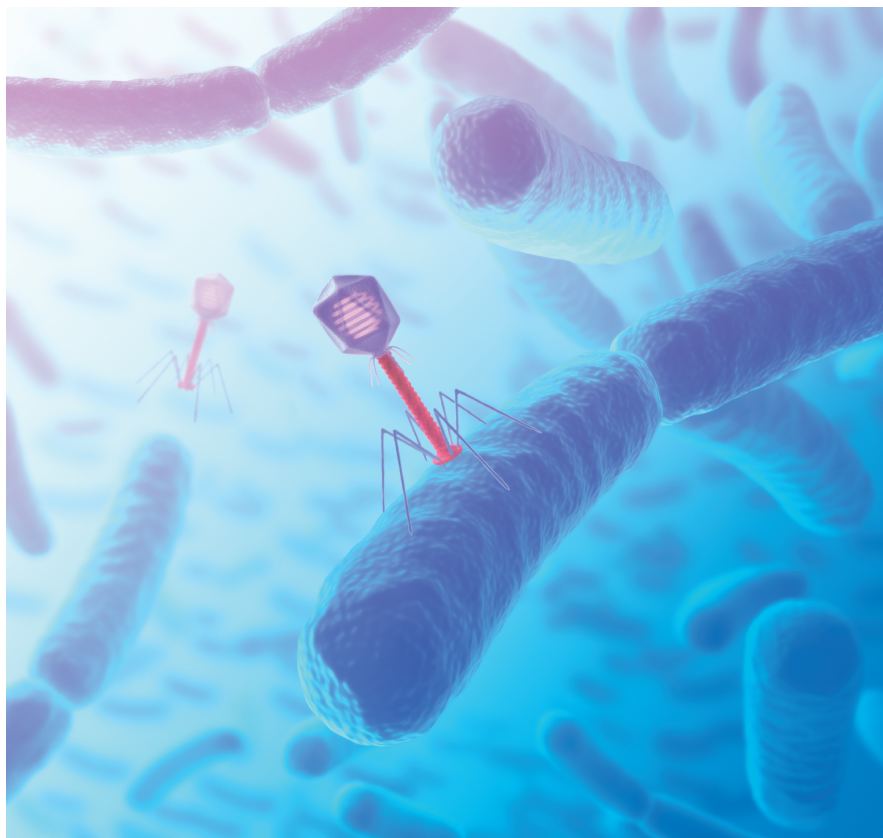
Rohwer, for example, has taken a census of the viruses inside the lungs of cystic fibrosis patients, and believes that they could influence the severity of the condition's symptoms (1, 2). Cystic fibrosis is caused by a mutation in the gene encoding a protein that regulates the body's mucus,

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digestive juices, and sweat, but Rohwer thinks that patients' complement of viruses, and when they acquired them, also plays a major role in the health of their lungs. "Every patient has a unique phenotype," he says. "Every patient also has a unique virome."

Other researchers are investigating viromic links to diseases including asthma, irritable bowel syndrome, Crohn's Disease, and even heart disease and diabetes. For now, however, translating these early findings to discoveries that would impact the clinical diagnosis or treatment of these diseases is far off, not least because so little is currently known about the make-up of the virome.

In June 2012, scientists around the world simultaneously published a series of papers spearheaded by the US National Institutes of Health's Human Microbiome Project (HMP) that characterized the fundamentals



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Researchers have found that many of the viruses in the human gut are bacteriophages—like the six-legged structure shown here attached to a rod-shaped bacterium—and likely impact health indirectly through their effects on the bacterial population.

of the microbiome in healthy individuals (3). By definition, the microbiome includes all microbes in the human body: bacteria, viruses, and fungi. Most initial HMP research, however, focused on bacteria because there is a standardized and thorough protocol for isolating and characterizing bacterial genes from the slurry of DNA in human feces or saliva swabs.

Viruses, in contrast, have so far been the forgotten siblings of the microbiome family. But a growing cadre of researchers argues that the human virome is probably at least as important to human health as our bacterial inhabitants.

Some viruses infect human cells, cause diseases, and then disappear; others leave lingering genetic signatures in the cells; and some can infect cells without causing symptoms at all. But many viruses found in the human body aren't hosted by our cells. Instead, they're renting rooms from the body's bacteria. "The great majority of viruses in the human body are going to be viruses that infect bacteria," says microbiologist Fred-eric Bushman of the University of Pennsylvania School of Medicine, Philadelphia, "And many of those are probably impacting humans by influencing the bacteria's functioning or abundance."

"There's this very complex ecology going on," adds immunologist Larissa Thackray of Washington University in St. Louis. Viruses that infect bacteria—known as bacteriophages, or just phages—"are probably the biggest regulators of the bacterial biomass," she says. Perhaps viruses would be better cast not as the forgotten member of the microbe family, but as bacteria's bossy older sibling.

Taking a Census

Human virome research has lagged behind the HMP's investigations into our bacterial hitchhikers for one major reason: It is much harder to identify viruses than bacteria.

All bacteria have a stretch of DNA called 16S rDNA that encodes a component of its ribosome, a cellular machine that produces proteins. Scientists trying to study the bacteria in a mish-mash of cells, and DNA can use a probe for 16S rDNA to fish out these bacterial genes. The precise sequence of DNA bases in the 16S gene is unique to every bacterium, so researchers can use the genes to compile a census of all the bacteria in the sample. For viruses, however,

there is no parallel to 16S rDNA.

"I think it's going to be very, very hard to find one assay that covers all viruses," says Thackray. To even isolate all the viruses in a single family, such as noroviruses, scientists must use two different primers—the molecular bait that sticks to particular DNA sequences—in order to isolate them, Thackray says.

So most researchers have turned to a different approach: shotgun sequencing. This involves sequencing every bit of DNA in

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the sample, whether it's human, bacterial, or viral. Computational biologists can then use powerful data analysis programs to sort through the massive number of sequences and find ones that resemble known viral genes. The main problem is that if a virus doesn't have a sequence similar to any known viral genes, it won't show up in the search. "We don't know how much we're missing right now," says Rohwer, "but we know that every time we change techniques, we find new viruses."

Bushman is trying a different tack. He purifies viruses while they're still intact, rather than trying to isolate their DNA from a mix of genes, by sifting samples of human feces through size-filtration and density-screening steps so that only virus-like particles remain. When the sample is clean, he breaks open the viral particles and sequences the genes they contain. "We know even then we are missing some," says Bushman. "It's a slice of the viral community, not the whole thing." When he applied these methods to samples from just 12 people, "the vast majority of viruses were new," he says. Still, every newly discovered virus helps improve the search for additional viruses by swelling the databases that computational approaches rely on.

Using shotgun sequencing, researchers have already made fundamental discoveries about variation in the human virome. For example, different places in the body harbor different viromes, differences in people's diets can be linked to virome variation, and mothers and identical twins

tend to have similar viromes (4, 5).

Thackray and her colleague Herbert "Skip" Virgin of Washington University School of Medicine, St. Louis, have also shown how the virome can play a complicated dance with the genome to affect disease. They discovered a virus that caused no symptoms in most mice that it infected, but mice with a particular version of a gene—a susceptibility allele—developed symptoms similar to human irritable bowel disease (6). "You can have this susceptibility allele and not get disease," says Thackray, "But if you have the allele as well as exposure to this virus then we see the pathology."

A similar model might be involved in asthma, says immunologist Akiko Iwasaki of Yale School of Medicine, New Haven, CT. Growing evidence suggests that although asthma is largely a genetic disorder, many children suffering asthma flare-ups have rhinovirus infections. "I think this is just the tip of the iceberg," says Iwasaki. "For every currently unexplained disease there could be a virus or class of viruses associated with the disease." In some diseases, this might not mean a virus is causative, but that the viruses in someone's body impact symptoms or severity. This also seems to be the case in Rohwer's studies of cystic fibrosis.

Beyond Health and Disease

For now, researchers' understanding of the virome is too nascent to be used therapeutically. "We can get a clue of what's there now, and we can maybe manipulate it in the lab," says Rohwer, "but we can't predict whether someone would be better off eating a cocktail of viruses or not."

A more immediate goal would be to improve viral diagnostics, says microbiologist Kristine Wylie of Washington University School of Medicine, St. Louis. Wylie was a member of the team that spearheaded one of the few pieces of virome-related research included in HMP's collection of publications. She and her colleagues characterized the viruses in nose swabs from 176 children, some of whom were healthy and some with unexplained fevers. On average, they found, the children with unexplained fevers had more viruses than healthy kids, suggesting that viruses, and not bacterial infections, caused these fevers (7). Developing a test to identify such viruses could help cut back on unnecessary treatments, says Wylie. "Most

viruses can't be treated, you just wait it out," she says, "But some viral infections are not clearly diagnosed as viral right now. We hope that in the future if there's a better test to inform a clinician whether an infection is viral or bacterial, that could help limit antibiotic usage."

Changes to the virome could also serve as early indicators of changes to the bacterial part of the microbiome, says Rohwer, "You see the virome change more quickly than the bacteria or the human phenotype," he says, "And there are much more of them so the signal is also amplified."

Studying the virome could even improve drug development. If the viromes of lab mice and rats differ significantly from

humans, does that make these animals poor models of human disease? "A lot of the mouse models we rely on for biology are infected with all sorts of viruses," says Thackray. "It makes you wonder whether this explains some of the variability we see in drug trials between mice and humans."

As researchers tackle these questions, it seems that the virome's profile can only continue to grow. "It's true that it's an overlooked part of the microbiome," says Wylie. "It would be nice if people think of the viruses in their bodies as well as bacteria when they hear about the microbiome."

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2 Willner D, et al. (2012) Spatial distribution of microbial communities in the cystic fibrosis lung. *ISME J* 6:471–474.

3 Relman, DA. (2012) Microbiology: Learning about who we are. *Nature* 486:194–195.

4 Minot S, et al. (2011) The human gut virome: Inter-individual variation and dynamic response to diet. *Genome Res* 21:1616–1625.

5 Reyes A, et al. (2010) Viruses in the faecal microbiota of monozygotic twins and their mothers. *Nature* 466:334–338.

6 Cadwell K, et al. (2010) Virus-plus-susceptibility gene interaction determines Crohn's disease gene *Atg16L1* phenotypes in intestine. *Cell* 141:1135–1145.

7 Wylie KM, Mihindukulasuriya KA, Sodergren E, Weinstock GM, Storch GA. (2012) Sequence analysis of the human virome in febrile and afebrile children. *PLoS One* 7(6):e27735.