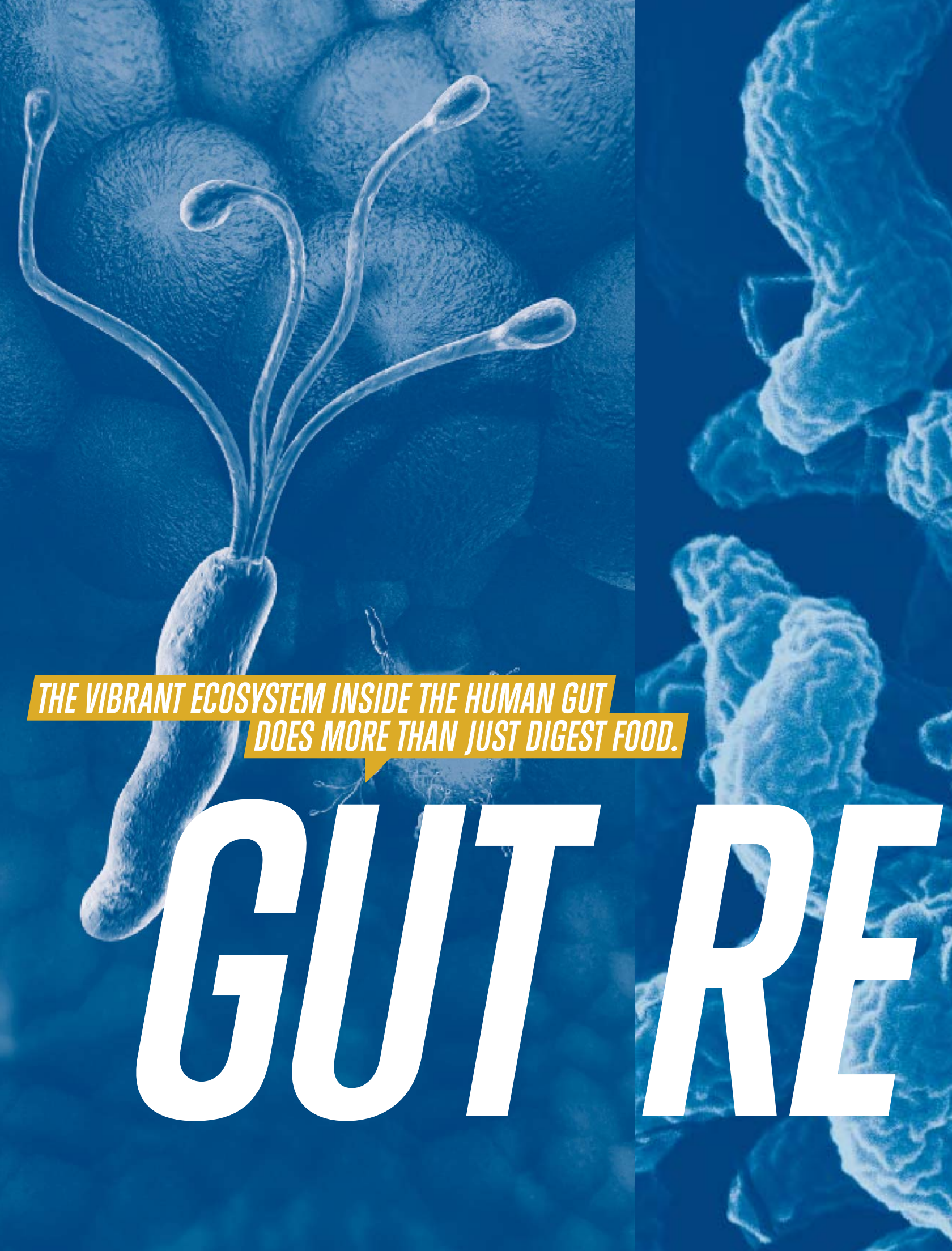


THE VIBRANT ECOSYSTEM INSIDE THE HUMAN GUT DOES MORE THAN JUST DIGEST FOOD.

GUT RE

ACTION

BY SARAH C.P. WILLIAMS

THE

genetics of the fat and skinny mice: identical. Their ages: all the same. The food in front of them: the same bland pellets in every cage. But the billions of bacteria teeming through each mouse's intestines: vastly different.

The variation in gut bacteria between lean and obese mice isn't just a consequence of their health, it's a cause, according to work by Rob Knight, an HHMI early career scientist at the University of Colorado at Boulder, Emory University pathologist Andrew Gewirtz, and their colleagues. Transferring gut bacteria from obese mice with insulin resistance and metabolic syndrome to healthy mice makes the healthy mice develop metabolic syndrome, they found. Giving the mice antibiotics before the bacterial transfer can prevent the syndrome. Moreover, the researchers discovered that the changed microbiota doesn't just affect molecules inside the mouse gut, it also affects outward behavior: the mice eat more than their healthy counterparts who didn't receive transferred bacteria.

"Out of all our studies on obesity and microbes, this was the most shocking thing to me," says Ruth Ley, one of Knight and Gewirtz's collaborators in data analysis at Cornell University. "To have microbes actually affecting behavior."

These researchers and a growing number of other scientists study the microbiota—the microbes that make our bodies their homes. While bacteria call to mind disease-causing germs that require antibacterial soaps and drugs to exterminate them, the bacteria in healthy intestines are relatively tame. In fact, animals from snakes to mice to humans wouldn't survive without them. They turn food into energy, synthesize vitamins that their hosts' bodies can't produce, and engage in a complex and vital interplay with the immune system.

Researchers are also showing that the composition of a person's microbiota can predispose him or her to diseases—from asthma and allergies to cancers, infections, and inflammatory bowel disease.

"Our individuality is not just us genetically, it's us plus all the microbes we carry," says HHMI investigator Ruslan Medzhitov of the Yale School of Medicine, who also studies the microbiota. "And that's not just a metaphor; they really shape our biology in many ways."

While the studies of Knight, Gewirtz, and Ley hint at the importance of the microbiota in human health, scientists have more questions than answers. After all, most of the bacteria swarming in our guts remain unidentified. Many cannot survive outside the complex environment of the intestines and so are difficult to culture in the lab. Plus, the microbiota of any one person is shaped not only by genetics but also by diet and environment, meaning that they change over time, complicating experiments even more.

CENSUS TAKERS

Knight's background is in ecology, studying the interplay of plants and animals in natural environments. He views the human body as another environment to study, in the same way he would study a jungle, marsh, or arctic tundra. Except, he says, bacteria are a lot more convenient to study. "Instead of having to do field seasons every year for a decade, you can draw out four quadrants on your forearm and have your field season in your office in fifteen minutes."

The first thing an ecologist does in an environment is take a census of what's there. So that's Knight's first task when it comes to the intestines, and it's the goal of microbiota researchers worldwide. Rather than a butterfly net and microscope, they use cutting-edge DNA sequencing technology to get snapshots of the genetic makeup—called the microbiome—of individuals' particular microbial mixtures.

Knight takes samples of bacteria—from skin, feces, and the intestines of mice or humans—and sorts out every copy of one particular gene encoding a bacterial ribosome. The ribosome is the cellular factory that produces proteins. It varies enough among bacteria that its sequence can place a bacterium (even a previously unknown one) into what's called a phylotype—essentially, "a spot on the tree of life," says Knight. Similar ribosomes are from similar bacteria and therefore appear on nearby branches of the tree. Knight originally developed a computational method, called UniFrac, to study the ribosomal differences among bacterial communities in sediment, ice, and water. Now, his team is using the same technique to tackle medical questions.

In a 2009 *Science* paper, Knight's lab group reported using UniFrac to analyze bacteria from 27 sites on the bodies of nine individuals, collected on four different dates. He found that bacterial communities varied drastically from person to person and changed to a lesser extent in one person over time. Only 3 percent of bacterial phylotypes appeared in all individuals on all occasions. In a separate paper, Knight

Previous pages: Helicobacter: *dieaktivisten.de/UFZ* E. coli and Campylobacter: *Creative Commons*

and collaborators showed that people's skin bacteria differ enough that you leave behind unique molecular fingerprints on everything you touch—a finding that could change forensic science.

While Knight's initial goal is to use UniFrac to characterize the microbiomes of healthy individuals, he says it will take the field only so far. Significant findings in ecology often come not from studies of healthy environments but from observations of disturbances within a community.

"If you went to Yellowstone and ground up a cubic mile of it and analyzed the DNA, you wouldn't find a lot of wolf DNA," says Knight. But scientists know that wolves are a species crucial to maintaining Yellowstone's diversity because of the radical changes that took place when they were removed from Yellowstone in the early part of the 20th century. Elk populations skyrocketed and the condition of the woodlands deteriorated. In 1995, scientists reintroduced wolves to Yellowstone and have since observed the elk population stabilize at a healthier number.

In the gut, Knight says, there could be bacteria that, while not abundant, keep the populations of other microbes in check.

With that in mind, he wants to use UniFrac to observe the complicated dynamics between human health and the diversity of phylotypes in the gut.

SEA OF MICROBES

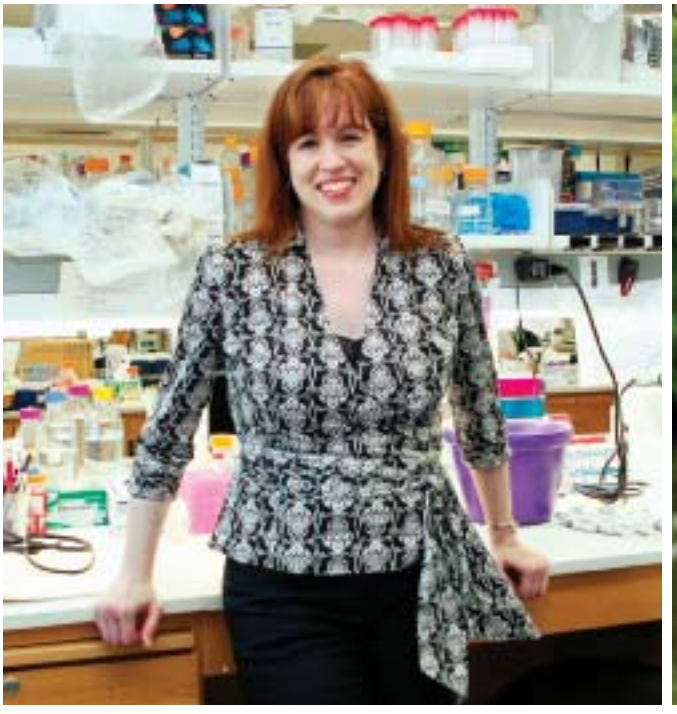
Martin Blaser, a doctor of infectious diseases and chair of medicine at New York University Langone Medical Center, thinks there's already a disturbance going on in human guts that's not unlike the disappearance of wolves from Yellowstone. From early human history until the 20th century, Blaser says, a bacterium called *Helicobacter pylori* was universal.

"You can actually trace human migration by looking at variations in *Helicobacter*," he says. Today, *Helicobacter* is still ubiquitous in developing countries around the world. In the United States, though, fewer than 6 percent of children have *Helicobacter* in their mix of gut bacteria. And in a series of recent studies, Blaser showed that children lacking *Helicobacter* are more likely than their peers to develop child-onset asthma. While the presence of *Helicobacter* predisposes people to ulcers, he thinks the disappearance of the microbe could explain not only rising rates of asthma but also allergies and obesity.

RUSLAN MEDZHITOV AND BRETT FINLAY ARE INVESTIGATING HOW CHANGING A PERSON'S MICROBIOTA IMPACTS HEALTH.

Medzhitov: Chris Jones Finlay: Birthe Piontek

LORA HOOPER AND ROB KNIGHT HAVE APPLIED AN ECOLOGICAL APPROACH TO STUDYING THE HUMAN GUT.



"We evolved in this sea of microbes, and now we're living as germ-free as we can," agrees Brett Finlay, an HHMI international research scholar at the University of British Columbia. "It's a major light going on right now, that this could have consequences."

Blaser says microbiota research is at the cusp of a scientific revolution and touching medicine at all its edges. "It's permeating so many different fields," he says. "In my role in medicine, I talk to nephrologists about the link to kidney disease; I talk to oncologists about the link to cancers. And I think we're going to keep finding new links to diseases."

One disease increasingly linked to the makeup of the gut microbiota: colitis, an inflammation of the colon. Tom Schmidt, a microbial ecologist at Michigan State University, is collaborating with doctors at the University of Chicago to study this connection when it comes to one particular form of colitis.

When doctors remove someone's colon—because of infection, weak spots, or cancer—they replace it with a new colon, built from other nearby tissue. At first, this pouch is void of bacteria. Gradually, a microbial community develops. But in almost half of all pouches, symptoms of colitis develop. So Schmidt and his collaborators are following patients with new colons and tracking the development of the microbiota in each case. They hope to discover how the intestinal flora keeps some colons healthy and others prone to infection and inflammation.

Schmidt, like Knight, comes at the microbiota with an ecology background. His expertise is in soil ecology, and soil has surprising similarities to the gut, he says. Both environments are low in oxygen. So the techniques he developed to cultivate organisms that thrive in low-oxygen soil he can now apply to the gut. And the big question that Schmidt hopes to answer resonates with both environments.

"It's a fundamental ecological question: how resilient is this community?" says Schmidt. "In soil, we look at what happens after you change the land from agriculture to abandoned, or from grassland to agriculture. In the gut, we look at what happens after a course of antibiotics, or in a new colon. How quickly can the community recover to its previous state? Does it recover at all?"

Finlay has some of the same questions. He wants to know how antibiotics change the bacterial community in the gut and how this shift can lead to, or prevent, disease. He uses techniques similar to Knight's to get a snapshot of a mouse's microbiome. Then he gives the mouse an antibiotic and takes a new snapshot. One study, by another lab group, showed that pretreating mice with antibiotics shifted their gut microbes so that they became resistant to *Salmonella* infection. Other findings, by Finlay and his colleagues, suggest that shifts in the microbiota caused by different antibiotics can weaken the immune system.

"When researchers compare mice with different degrees of susceptibility to disease, they've always searched the mouse genes for the explanation and not found much," Finlay says. "Now we're learning that's because the difference isn't in the mouse genes, it's in the microbiota."

NOT SO BLACK AND WHITE

In any kind of census, it's necessary to group individuals into categories that oversimplify their differences. In a human census,

Hooper: Amy Gutierrez / AP, ©HHMI Knight: Carmel Zucker

that means checkboxes that reduce people to race, gender, income, and marital status. In the microbiota census occupying scientists' minds, it means the temptation to group gut bacteria into good and bad, pathogens versus commensals (microbes that don't cause disease). But this process hardly paints a full picture of what's going on in our intestines, says Yale's Medzhitov.

"The difference between commensals and pathogens is not that they are two very different types of microbes," Medzhitov explains. In fact, microbes considered commensals in one organism's gut, or in one situation, can act pathogenically in a host with a compromised immune system or in a different organism. "So the distinctions between the two are in many ways arbitrary," Medzhitov says.

In reality, each bacterium in our gut falls along a spectrum between pathogenic and commensal. Medzhitov thinks that instead of lumping bacteria into these extremes, based on their outcomes (disease or health), scientists should focus on how each bacterium interacts with its host—the human body.

Humans have intricate immune systems exquisitely tuned to identify intruders. Medzhitov studies a class of receptors—called Toll-like receptors—that recognize invading bacteria and signal the immune system to act. His lab has found that Toll-like receptors recognize bacteria that could do harm to the body, and they also help keep the intestinal microbiota balanced by detecting bacteria that are less virulent.

"There's something about the environment of the gut that controls this interaction so that, under healthy conditions, Toll-like receptors sense commensals and don't react to them as harmful," says Medzhitov. Of course, if those same tame bacteria sneak out of the gut into the bloodstream—during surgery, for example—the receptors will respond with fury, leading to dangerous inflammation and sepsis. Medzhitov wants to know what it is about the intestines that keeps Toll-like receptors in check.

And that's far from the only way the immune system interacts with gut bacteria. HHMI investigator Lora Hooper, at the University of Texas Southwestern, is fascinated by the subtleties of the interaction.

"I've been studying this for 15 years and it's still not clear to me—how can you have a hundred trillion bacteria in your gut and you don't get sick?" says Hooper.

When she first started studying the microbiota, as a postdoctoral fellow in the lab of Jeffrey I. Gordon at Washington University in St. Louis School of Medicine, Hooper began working with germ-free mice. These mice are raised from birth in sterile environments—they eat sterilized food, live in germ-free bubbles, and interact only with other germ-free mice. These cleaner-than-clean mice allow researchers to study the effects of individual bacteria strains in a simple system.

Hooper and Gordon's first experiments with germ-free mice gave them a glimpse at some of the jobs of gut bacteria: they stimulated immune responses, helped detoxify compounds the mice ate, stimulated the growth of new blood vessels, allowed proper tissue development, and performed countless metabolic tasks.

When she moved to her own lab, Hooper took another look at the plethora of genes that shot up in expression levels when a mouse was first exposed to gut bacteria. She chose one to study in more depth. Her lab quickly discovered that it was the gene for a protein dubbed RegIIIγ, and it had a rare job for a protein: it's an antibiotic. She's gone on to show that RegIIIγ can kill bacteria by drilling a hole in their outer layers, allowing their contents seep out. Hooper speculates that RegIIIγ may help to shield the intestinal epithelium from the bacteria sloshing around inside the gut.

"The epithelium itself is a barrier, but you want to minimize even having bacteria attach to that," says Hooper. "So these antimicrobial proteins, and probably many other immune molecules, likely help to set up a secondary barrier."

In patients with inflammatory bowel disease, more bacterial cells reach the intestinal lining, indicating that the chemical barrier is inadequate—one hint toward a cause of this chronic disease.

PERSONALIZE THE GUT

Though there's a growing body of evidence that links variations in the microbiota to diseases—from Knight's studies on metabolic syndrome to Blaser's work on asthma and Schmidt's colitis research—the mechanisms of these links are still too sketchy to translate them into clinical medicine.

A 2009 paper in the *Proceedings of the National Academy of Sciences* by Jeremy Nicholson at Imperial College London and collaborators at the drug company Pfizer found that variations in one type of gut bacteria lead to differences in how people metabolize acetaminophen (Tylenol) and different propensities for liver toxicity. The authors proposed that "assessing the effects of microbiome activity should be an integral part of pharmaceutical development and of personalized health care."

The concept resonates strongly with Knight. The vast variation he's seen between individuals' microbiomes leads him to think that gut bacteria will be targeted with drugs, or personalized concoctions of healthy bacteria, in the future.

"In terms of developing personalized medicine," he says, "it seems like it makes more sense to develop medicines based on the microbiome, where the variation is so great, rather than on the human genome, where the variation is so little."

For now, though, patients and doctors are stuck at the impasse between knowing (or guessing) the cause of a disease and having a treatment. Someone can blame their diabetes or inflammatory bowel disease on the churning mass of bacteria that lives inside their intestines, but there's no magic pill to change the dynamics of that complicated world of the human microbiome. ■