MICROBIOLOGY

Simple Hosts May Help Reveal How Bacteria Infect Cells

Easily studied organisms such as fruit flies, worms, and yeast are turning out to be good hosts for bacteria that cause human diseases

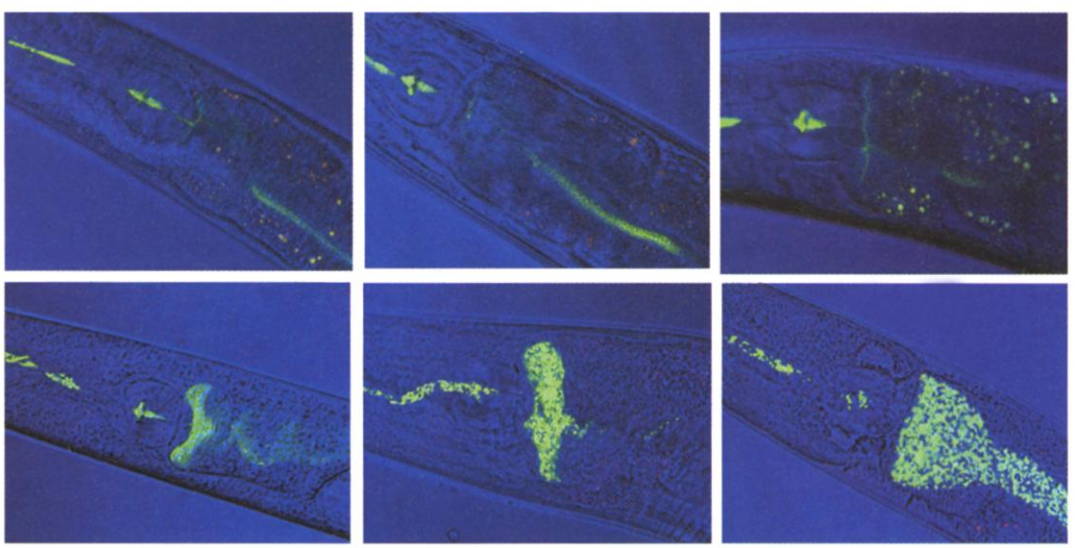
When microbiologist Laurence Rahme began speculating in the early 1990s that some human pathogens might infect plants, "a lot of people looked at me in a very weird way," she recalls. And when she suggested that the infected plants might respond—at least on a molecular level—in ways that mimic those of sick people, "people told me I was crazy." After all, plants don't cough or throw up. Given the physiological gulf between plants and people, the whole notion seemed far-fetched.

But Rahme persevered, encouraged by her thesis adviser, Nickolas Panopoulos of the University of California, Berkeley, and a later mentor, geneticist Frederick Ausubel of Harvard Medical School in Boston. Today, she is at the vanguard of a new area of microbiological research: using not only plants but also other simple organisms—such as the roundworm *Caenorhabditis elegans*, the social amoeba *Dictyostelium discoideum* (sometimes referred to as slime mold), the fruit fly, and even yeast—to study the interactions between harmful bacteria and their hosts.

One indication of the field's newfound respectability came at this year's meeting of the American Society for Microbiology (ASM),* which held its first-ever session on the topic. "I can't tell you how many people have told me how they're going to test worms with their [infection] model," says Colin Manoil, a geneticist at the University of Washington, Seattle. "This field is in its infancy, but it's going to become huge."

Driving this work are findings that support Rahme's initial speculation: Important human pathogens—including *Pseudomonas aeruginosa*, a common cause of infections in burn victims and cystic fibrosis patients, and *Legionella pneumophila*, which causes Legionnaire's disease—invade and harm simple organisms. What's more, these infections require many of the same bacterial genes needed to make mammals sick. These observations suggest that even though simple organisms aren't perfect models for complex hosts—in particular, they lack the highly specific immune responses that mammals deploy when fighting off microbes—the basic mechanisms by which bacteria establish infections in the various organisms may be similar.

And that, in turn, suggests that the unconventional hosts may help researchers explore what microbiologist Samuel Miller, who's also at the University of Washington, Seattle, calls "the real big frontier"—the mammalian side of bacterial invasions. Identifying the host proteins that either promote infections or help ward them off would not only shed light on the basic mechanisms of infectious diseases but also provide potential new targets for antibacterial drugs.



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Model infection. When *C. elegans* worms feed on *S. typhimurium*, the bacteria proliferate in the animals' intestines, causing marked distention (bottom panels). That does not happen in worms feeding on nonpathogenic *E. coli* bacteria (top panels).

Promising parallels, powerful genetics

Progress in identifying those host proteins has been slow, mainly because the standard models for human diseases—usually mice or mammalian cells in culture—are so genetically unwieldy that they don't readily lend themselves to the experimental analyses needed to do the job. But such studies are relatively easy to carry out with genetically tractable animals such as yeast, worms, and fruit flies.

Indeed, scientists have appreciated the power of those organisms for decades. So it might seem surprising that only now are significant numbers of laboratories using them to study pathogenesis. "It's one of those things where once it starts happening, you wonder why it didn't happen before," says Manoil. As Rahme found when she made her original proposals, however, many investigators simply weren't convinced that the approach would work. "I don't think people realized that there would be so many conserved features of pathogenesis," says Ausubel. But Rahme says that when she shared her thoughts with the Harvard geneticist before joining his lab as a postdoc in 1992, she found him unusually open minded. "I thought it was a long shot, but a great idea," says Ausubel.

Rahme's Berkeley adviser, Panopoulos, had told her about several little-known discoveries made 15 to 20 years earlier, suggesting that some strains of *P. aeruginosa* cause illness in both plants and humans. Those experiments had been performed on celery, lettuce, and potato plants, but Rahme wanted to study a plant with a genetic makeup that was better understood and more easily manipulated. So, she sifted through a collection of 75 *P. aeruginosa* strains, looking for any that would sicken the well-studied plant, *Arabidopsis thaliana*.

By 1995, Rahme had found two bacterial strains that fit the bill—they rotted *A. thaliana* leaves—and she decided to focus on the one that had originally been isolated from a burn patient. She found that mutants that did not produce proteins known to be important for virulence in

* 21–25 May in Los Angeles, California.

mice were harmless to plants, and vice versa. The observation was crucial, because it indicated that infections of plants and mammals share common features (*Science*, 30 June 1995, p. 1899). Buoyed by this result, Rahme and her colleagues went on to search for other host models for studying *P. aeruginosa* pathogenicity.

A graduate student in Ausubel's lab, Man-Wah Tan, identified one: *C. elegans*, which died when fed the bacteria. The researchers again found a large degree of overlap between the *P. aeruginosa* genes needed for pathogenesis in plants, mice, and worms. "That established very firmly that the underlying mechanism of pathogenesis was very similar," says Ausubel.

Still, some scientists remained skeptical of the general applicability of using unconventional hosts to study bacterial infections. *P. aeruginosa* is "a nonspecific pathogen by nature," claims Jeffery F. Miller, a microbiologist at the University of California School of Medicine in Los Angeles. "Other organisms are more picky, colonizing only particular tissues and cell types through the use of specific associations between host and bacterial molecules."

At the ASM meeting, however, Ausubel reported that Alejandro Aballay and Peter Yorgey, postdocs in his lab, had found that *Salmonella typhimurium*, a bacterium with a more restrictive host range—it normally targets animals with backbones—can infect worms, setting up shop inside the gut and eventually killing the animals. Some of the results hint that the microbe binds to a molecule or molecules in the worm intestine. In contrast to *P. aeruginosa*, "once *Salmonella* are in there and proliferating, you can't wash them out," says Ausubel. (The results were published in the November issue of *Current Biology*.)

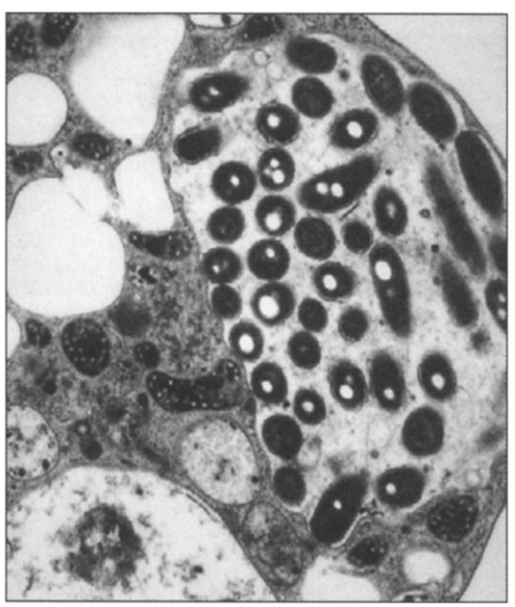
Salmonella's ability to stake out the intestine in this way may represent some very conserved aspect of the infection process, Ausubel suggests. "Certainly, molecular interactions between specific host and bacterial factors have been honed by evolution, and many of these are critical to see the full effects of *Salmonella* infection in its natural host," he says. "But maybe the first step—the ability to colonize—is more ancient than we'd thought."

The early criticisms notwithstanding, the Ausubel team's initial discoveries caught the eye of other investigators, including Ralph Isberg, a microbiologist at Tufts University School of Medicine in Boston. He had toyed with the idea of exploiting simple hosts for a long time and had made some pilot efforts with *Legionella* that hadn't panned out. But when Isberg saw what Rahme and Ausubel had done with *Arabidopsis*, he recalls, "I thought, 'That's a really important experiment.' They screened dozens of *Pseudomonas* strains, and only two gave striking disease. That said we shouldn't give up after trying just one or two strains of *Legionella*." His group went back into the lab with a new set of strains, determined to find one that would grow in the genetically tractable organism *D. discoideum*.

Other groups, like Ausubel's and Manoil's, were looking at the worm, and still others were turning their attention to additional simple creatures. For example, Washington's Samuel Miller thought he could speed up his progress if he used yeast. During his postdoc, while he was working with mammals in a different field, he heard a talk by a yeast geneticist, and he remembers "being completely blown away, thinking, 'They're going to stomp on me.' The mammalian system is just so slow."

Co-opting host molecules

Researchers are just beginning to exploit the power of these experimental systems to gain new insights into host-pathogen interactions. "You don't just start one of these models and hit a home run," says Manoil. Even so, they already have evidence that the simple organisms can help them identify host proteins that facilitate bacterial infection.



Legionnaires for amoebae. Dozens of dark-staining *L. pneumophila* bacteria can be seen in the phagosome of this *D. discoideum* cell.

For example, Manoil and Creg Darby, then a graduate student in Manoil's lab, identified a *C. elegans* gene involved in susceptibility to a then-unidentified *P. aeruginosa* toxin. This poison paralyzes worms, so Darby looked for animals that could move after spending several hours in bacteria-covered culture dishes. As reported in the 21 December 1999 issue of the *Proceedings of the National Academy of Sciences*, Darby found two mutants, each of which carried defects that mapped to the same gene, called *egl-9*.

Although the *C. elegans egl-9* gene is known for its role in egg laying, both rodents and humans carry a related gene. No one knows what the human version does, but the one in rodents triggers programmed cell death, a form of cellular suicide that eliminates stressed or damaged cells. If the human *egl-9* relative induces cell death in response to *P. aeruginosa*, it might be involved in the tissue damage associated with infections. If so, Manoil and Darby may have laid the groundwork for finding a drug that blocks a host target. "In mice, you can take something you think is involved and test it, even though that takes a lot of work," Manoil says. But with worms, "you can discover some new target that you'd have no notion of otherwise. This lets the organism tell you what's important."

Samuel Miller and colleagues are using yeast to study the reactions of host cells to proteins produced by *S. typhimurium* and *Yersinia pseudotuberculosis*, both of which cause gastroenteritis in humans. These organisms belong to a class of bacteria that use "molecular syringes" to shoot particular proteins into host cells (*Science*, 31 May 1996, p. 1261). There, the proteins harness host cell molecules and put them to work for the bacterium's benefit. Yeast geneticist Cammie Lesser, a postdoc in Miller's lab, engineered strains of the yeast *Saccharomyces cerevisiae* to produce individual proteins that *S. typhimurium* or *Y. pseudotuberculosis* injects into human cells. At the ASM meeting, Miller reported that the *Yersinia* proteins target the same sites in yeast cells as they do in mammalian cells, providing a vote of confidence for this approach.

Lesser and Miller's results with a protein called SipA/SspA, which *Salmonella* bacteria inject into their target cells, were also encouraging. Last year, test tube studies by microbiologist Jorge Galán's group at Yale University School of Medicine suggested a possible role for the protein (*Science*, 8 January 1999, p. 167). This work showed that it braces the actin-based protein filaments that provide internal structure to the so-called ruffles that sweep the microbes into the cells. This and other observations led the researchers to hypothesize that SipA/SspA localizes the formation of the ruffles to the sites where bacteria bind. Miller and Lesser have now found that SipA/SspA performs tasks in living yeast cells similar to those postulated to

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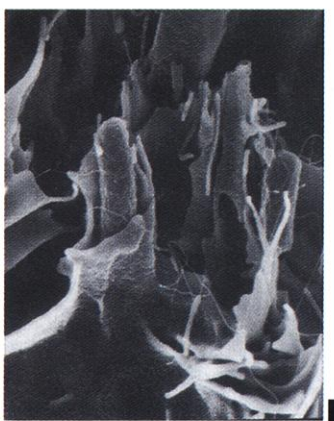
occur in mammalian cells, thereby linking processes observed with purified proteins to events in intact cells.

Next, the researchers hope to identify host proteins involved in these bacterial infections. Because these particular *Yersinia* and *Salmonella* proteins kill or slow the growth of yeast cells, the researchers plan to screen for host proteins that rescue the cells when present at high concentrations or in altered versions.

Exploiting intact bacteria

Whereas some researchers are using individual bacterial proteins to ensnare host partners, others are employing whole bacteria. This approach may snag host factors involved in multiple aspects of infection, compared to those that interact with a specific bacterial molecule. Take Isberg's research on *D. discoideum* and the bacterium that causes Legionnaire's disease.



Individual *D. discoideum* cells resemble both *L. pneumophila*'s targets in the body—immune cells called macrophages—and the pathogen's amoeba hosts in habitats such as water towers. For example, all three types of cells slurp bacteria from their environments. Amoebae and *D. discoideum* consume the microbes for food, whereas macrophages demolish them to protect the host animal. But whereas macrophages and most amoebae are not amenable to genetic manipulation, *D. discoideum* is.

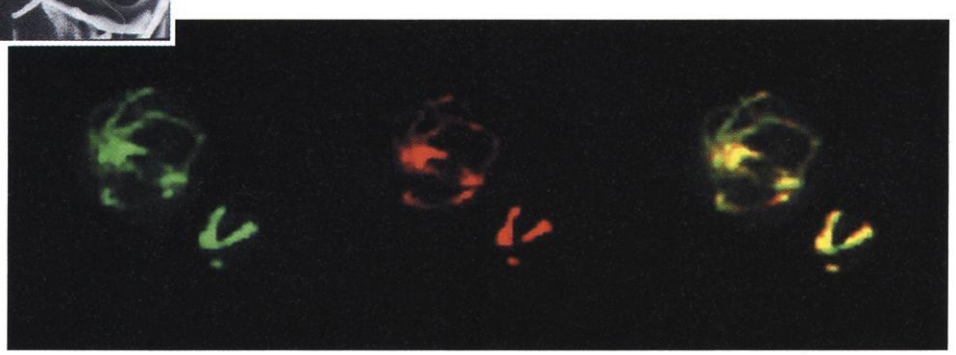
Jonathan Solomon, a postdoc in Isberg's lab, has found that when *D. discoideum* cells ingest *L. pneumophila*, the bacteria grow inside membrane-bound sacs, just as they do in macrophages and amoeba, until they eventually kill the cells. Solomon then went on to try to identify the *D. discoideum* genes needed for *Legionella* infectivity by examining the effects of known mutations on the growth of *L. pneumophila*.

One of his most interesting findings implicates a surprising protein in *Legionella* infection, Isberg says. Most bacteria inside macrophages travel within membrane-bound sacs that merge with other sacs until they reach the lysosome, which contains enzymes that destroy the bacteria. Scientists had thought that *L. pneumophila* dodges this fate by blocking fusion of the membranes in which the bacterium resides. Yet these studies, described in the May issue of *Infection and Immunity*, suggest that, for reasons not yet understood, the microbe might require a host protein that fosters—not inhibits—membrane fusion for its life inside *D. discoideum*.

Solomon is currently testing whether *L. pneumophila* relies on a similar protein in human macrophages. "That's where this approach is powerful," Isberg says. "You get unexpected information from this simple organism, and then you can go back and see whether the same thing's important in mammalian cells."

Host molecules that fight back

Whereas many researchers are seeking genes that facilitate infections, Ausubel has started to identify those that normally protect the host. Because both *P. aeruginosa* and *S. typhimurium* kill the worms they infect, Ausubel's group is now screening for worms that die unusually quickly, with the idea that these hosts harbor defective versions of proteins that normally minimize damage by the microbes. Although the susceptible worms die, their eggs can still be harvested and will grow into adults if placed on a fresh food source. The ability to find mutants with increased rather than decreased susceptibility to the microbes' fatal effects represents a unique strength of using worms, Ausubel points out.



Swept up. The yellow color produced by superimposing the green-tagged SipA/SspA protein from *Salmonella* on the red-stained cellular actin protein shows that the two proteins colocalize in yeast cells. This may aid formation of the ruffles that sweep *Salmonella* bacteria into the cells they infect (as shown at upper left).

But with all these studies, scientists miss pathogen-fighting innovations that have arisen later during evolution. Worms, yeast, *D. discoideum*, fruit flies, and plants don't produce the antibodies or T cells that respond to specific microbes, for example. However, work on the unconventional hosts may help reveal the workings of the so-called "innate immune system"—a nonspecific but widespread system for combating microbes that has been found in organisms ranging from plants to humans (*Science*, 25 September 1998, p. 1942). Kathryn Anderson, a geneticist at the Sloan-Kettering Institute in Manhattan, says it's very important to understand these core responses: "Frequently, the way the pathogen tries to fight the host is to attack these evolutionarily conserved pathways."

She and others are using a variety of approaches to identify components of the innate immune system. Anderson's group has found roughly two dozen genes involved in the fruit fly's innate response to infection by the bacterium *Escherichia coli*. Some are expected to be involved in resistance to bacteria, because they resemble genes known to play roles in innate immune system pathways in mammalian cells and mice. But others, she says, "haven't been defined in mammalian systems."

Rahme is taking a different approach to finding fruit fly genes involved in infection. Although *E. coli* infects fruit flies and stimulates a defensive response, it doesn't usually make them sick. Working with Kevin White of Yale University, Rahme is using DNA arrays to find genes whose activity changes when they are exposed to an overtly pathogenic bacterium, *P. aeruginosa*. After she has identified such genes—at least some of which she expects to be involved in defending against infection—she plans to disrupt them to find out whether they play a role in the fruit fly's response to the microbe. With a small group of promising candidates, she'll then make mouse knockouts to investigate the genes' role in mammalian defenses.

Studies of unconventional pathogen hosts have just begun to bear their first fruit, and no one yet knows how much impact they will have, but researchers are hopeful. These days, it's hard to find strong critics of the approach, says Rahme: "They changed their minds. Now they say, 'What a great system. I knew it was going to work.'"

–EVELYN STRAUSS

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