SIMPLE HOSTS

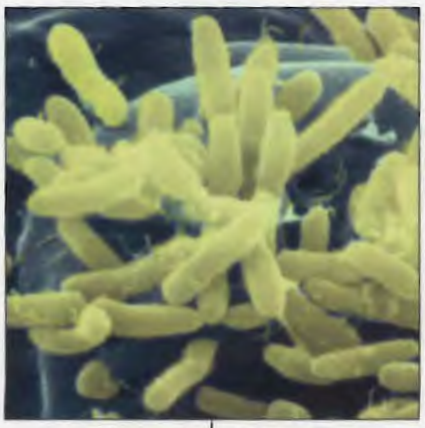
What plants, worms, and flies reveal about the complexities of human disease

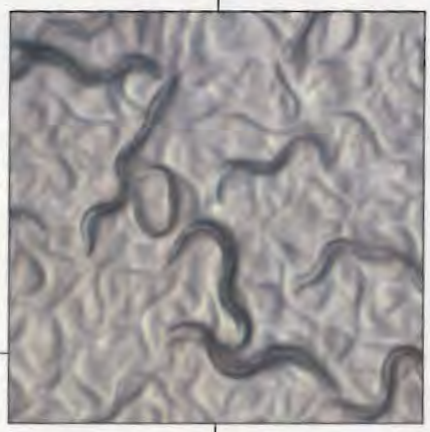
by PATRICIA THOMAS

THERE IS NO FLORIST in the lobby of Shriners Hospital for Children in Boston. In fact, anyone who attempts to deliver a get-well begonia or a mixed bouquet is sternly turned away. Soil—whether in a decorative pot or clinging to the stems of cut flowers—is very likely to harbor *Pseudomonas aeruginosa*, a bacterium that can cause life-threatening infections in the severely burned youngsters receiving treatment. Across the street at Massachusetts General Hospital, *P. aeruginosa* is the enemy not only of patients with burns, but also of those with major trauma or cystic fibrosis. When *Pseudomonas* colonizes the lungs, causing pneumonia, the likelihood of surviving is slim even at the best of hospitals, says surgeon Ronald G. Tompkins, chief of staff at Shriners, chief of trauma and burn services at MGH, and Burke professor of surgery at Harvard Medical School (HMS).

Although plants are banned from burn and trauma units, their study holds great promise for understanding, preventing, and treating the very infections that menace hospitalized patients. Researchers have used the tools of genetics and molecular biology to reveal amazing similarities in how *P. aeruginosa* and other pathogens infect plants, animals, and tiny creatures without backbones, and in how these embattled hosts fight back. At the vanguard of this effort at Harvard are a handful of maverick biologists who set out to defeat hunger during the "green revolution," but who have since turned their energies to pathogenesis and defense.

Only a decade ago, it was radical to think that simple hosts—plants and nonvertebrate animals such as nematodes and flies—had any utility in the study of human disease and immune response. Today, the use of simple models like these has been embraced by forward-thinking physicians like Tompkins and Stephen B. Calderwood '71, M.D. '75, chief of the infectious-disease division at MGH and a professor of medicine at HMS. The relevance of these models isn't immediately apparent to people who see humans as the center of the universe—after all, the differences in how plants and people respond to illness are obvious: people develop fevers, throw up, or take to their beds; plants remain rooted in place, dropping leaves or wilting. But there is another way to look at infections, Calderwood says. "From the bacterium's point of view, plants and humans are both environments where it needs to grow and protect itself. The biological problems it faces may not be that different."





Top: *Pseudomonas aeruginosa* bacteria on an *Arabidopsis thaliana* leaf. Middle: *Caenorhabditis elegans*, a flea-sized nematode, generates quickly and provides ready visual evidence of infection and virulence. Bottom: *Arabidopsis thaliana*, a mustard, was the first simple host in the groundbreaking infection research.

Several forces are pushing scientists to add unconventional models to old standbys such as mice, rats, and nonhuman primates. Laboratories are being inundated by raw data pouring out of genome-sequencing projects, and plants and simple hosts offer a faster and cheaper means for beginning to decipher this information. Their use also reduces the demand for larger animals, which may quiet some of the controversy that has roiled basic science in recent years. And biologists in the Faculty of Arts and Sciences (FAS) say that the bold attempt to model human disease in plants and tiny nonvertebrates complements their investigations of the evolutionary past.

For doctors, the need to know more about mechanisms of pathogenesis and defense is an urgent, life-or-death issue. Healthcare-related infections, primarily those picked up in hospitals, are a huge and costly problem that is becoming harder to treat as more bacteria develop antibiotic resistance. At Mass General, Calderwood estimates that 15 percent to 20 percent of all patients with *Enteracoccus* infections—including life-threatening bacterial endocarditis or sepsis—are carrying strains that shrug off the powerful drug vancomycin, once known as "the antibiotic of last resort."

PLANT: JOHN SOARES; WORMS: COURTESY FREDERICK AUSUBEL; BACTERIA: COURTESY JULIA PLOTNIKOVA AND FREDERICK AUSUBEL

Portraits by John Soares

THE SCIENTISTS WHO HAVE PIONEERED the use of plants as models for infection and defense won't be found on the wards of Mass General or other Harvard teaching hospitals. Instead, most have laboratories in the Wellman Building, a utilitarian, concrete-clad tower on the eastern edge of the MGH campus.

On the tenth floor of Wellman is the bustling laboratory of HMS genetics professor Frederick M. Ausubel, whose political idealism drew him into the world of plants some 30 years ago. He studied bacteria, like most geneticists of his generation, while earning his Ph.D. at the Massachusetts Institute of Technology. But when he graduated, in 1972, the field was shifting its attention from bacterial to mammalian genetics. Instead of following fashion, however, Ausubel turned to plants. He moved to Harvard to join forces with plant biologists who believed that they could feed the world by transferring the nitrogen-fixing ability of legumes to other crop plants, thus eliminating the need for costly, environmentally damaging, nitrogen fertilizer. They aimed to do this by taking nitrogen-fixing genes from beneficial bacteria, which lived symbiotically with legumes, and inserting these into major crop plants such as wheat or corn. But "nobody got it to work," Ausubel reports, "and people have largely abandoned this goal as technologically too complex."

Even as prospects for nitrogen fixation in edible plants dimmed, Ausubel and a number of other young geneticists kept working with plants, albeit not in an organized way. Because some studied maize while others focused on petunias, the genes they found were like pieces plucked from many different jigsaws and tossed onto a table where not even a puzzle master could assemble them into a coherent picture. Ausubel was certain that more progress would be made if geneticists all used the same

Frederick M. Ausubel's interests led him from the "green revolution" to unconventional studies of plant, and ultimately animal, infection.

model plant, but hardly anyone agreed with him. His idea gained currency, however, when it picked up support from Nobel laureate James Watson, head of the prestigious Cold Spring Harbor Laboratory on Long Island.

Although acceptance didn't come overnight, by the mid 1980s *Arabidopsis thaliana* had become the laboratory mouse of the plant world. In horticultural parlance, *Arabidopsis* is a "fast-cycling brassica," a type of mustard that belongs to the same family as broccoli, Brussels sprouts, and cabbages. It thrives in flats like those at commercial nurseries, but its small leaves and sparse yellow flowers would not get a second look from a gardener. Geneticists, on the other hand, don't care that *Arabidopsis* is homely. Short statnre makes it easy to grow and manipulate in the research greenhouse; a seed matures into a seed-producing plant in only three to six weeks; and the plant is self-fertilizing, making desirable mntations easier to perpetuate than they would be in a species that requires cross-fertilization.

In Frederick Ausubel's laboratory, researchers use the nematode *Caenorhabditis elegans* and the bacterium *Staphylococcus aureus*—treated so the bacteria fluoresce—to study virulence and host resistance to infection (detail at right).

Ausubel was an associate professor of biology in FAS in 1982, still studying the symbiotic relationship between nitrogen-fixing bacteria and plants, when he got an offer he couldn't refuse: Howard Goodman, who had recently founded the molecular biology department at Mass General, invited him to join the fledgling research center. As one of the first scientists to clone human genes for insulin and growth hormone, Goodman had gained considerable clout and an ample budget for the new department—much of it provided by Hoechst, a German pharmaceutical company since incorporated into Aventis, a drug industry giant. "That gave me the freedom to work on *Arabidopsis*, even though I didn't have any grants," Ausubel recalls.

Equipped with a new model plant, Ausubel began to ask different questions. It occurred to him that nitrogen fixation, a symbiotic relationship between bacteria and legumes, shared many features with pathogenesis: both involve a microbe and a host, and in each case the host recognizes and responds to the simpler organism. The difference is that one relationship is balanced and beneficial, whereas the other ends with the microbe running wild and damaging or killing the host. Once he resolved to explore infection in a systematic way, Ausubel decided to use a model system with *Arabidopsis* as the host and *Pseudomonas syringae*, a well-known bacterial pest specific to plants and closely related to *P. aeruginosa*, as the pathogen.

In the secret handshake of host and pathogen, Ausubel's lab focused almost entirely on what was happening on the host side. With funding from the U.S. Department of Agriculture and the National Science Foundation, Ausubel's team carried out detailed molecular and biochemical investigations of various plant genes and their products. The lab hummed along, generating publications and training graduate students. By the end of the 1980s, however, Ausubel had become interested in host responses in humans as well as plants, and was looking for a way to make his group's work more relevant to human health and disease.

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TRADITIONAL BOUNDARIES have never been impediments to molecular microbiologist Laurence Rahme (pronounced RAKH-mee), who spent her childhood in Greece and Lebanon, moved to Italy to study biology at the University of Naples, and traveled to the University of California at Berkeley for graduate school. With such a history, it is perhaps not surprising that this cosmopolitan scientist had a radical idea while working toward her Ph.D. in molecular plant pathology. Bacteria are equipped with virulence factors, items in a biological "toolbox" that they rely on to infiltrate and colonize their hosts. If researchers can see that a bacterium is using a screwdriver, rather than a drill, they have a better chance of blocking its action. When Rahme reviewed what was happening in the field of human pathogenesis, it seemed to her that scientists were bogged down because there was no standard method for identifying new virulence factors.

"To enrich the field, you need to find new players," Rahme explains. "How are you going to find new players if you don't have a system that will allow you to do that?" Biologists and physicians had noted since the 1930s that a few organisms, including the ubiquitous, soil-dwelling bacterium *Pseudomonas aeruginosa*, could infect both plants and humans—hence the ban on floral gifts in burn hospitals. But no one attempted to draw connections between the mechanisms of plant and human disease, because the assumption was that bacteria needed separate tools to penetrate the defenses of hosts that were so disparate. Rahme questioned this assumption, knowing that once Mother Nature finds a good tool she tends to use it over and over.

Biologists describe a gene or biological mechanism as "highly conserved" if it turns up repeatedly in creatures both simple and complex. The most highly conserved mechanisms are also thought to be the oldest. Evolutionary biologists say that the more ancient a piece of genetic code is, the more opportunity it had to be incorporated into new species as they branched off

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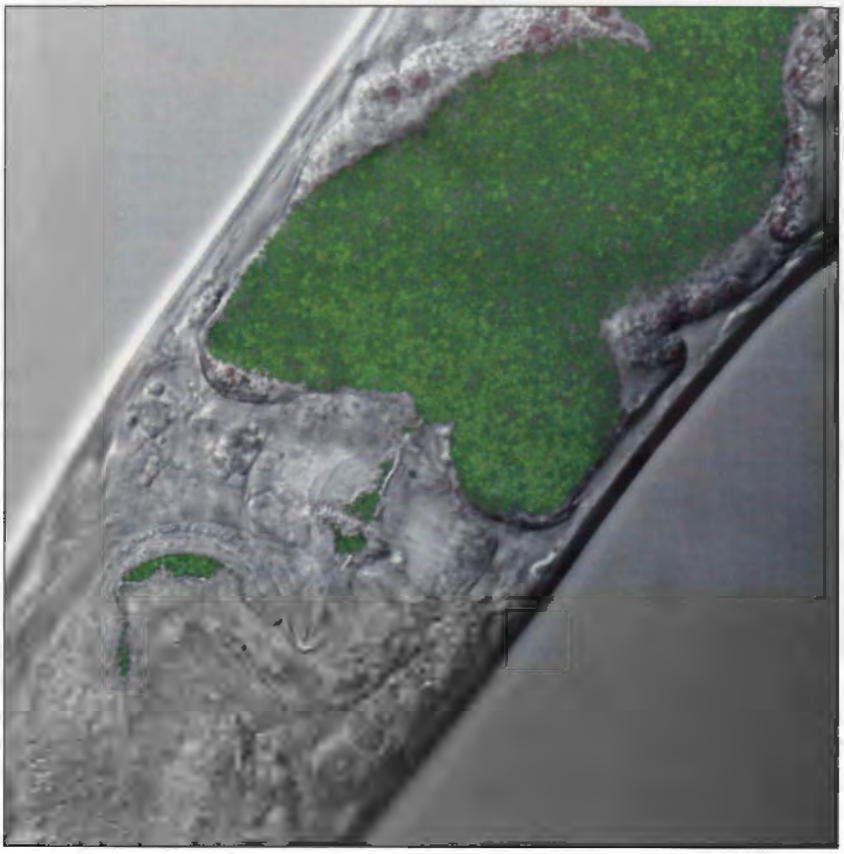
from older ones. More recent genetic developments, on the other hand, would not be passed along to disparate creatures evolving on other branches.

Rahme suspected that some virulence factors are very old indeed, and reasoned that, "if they are conserved, then you can use plants or any other simple system to start screening for these players in pathogenesis." She began work on *Pseudomonas* virulence factors at Berkeley, but had miles to go when she completed her degree in 1991.

Rahme wanted to continue her work in a lab where *Arabidopsis* was the main experimental organism, and Ausubel's operation was known as one of the best in the world. The prospect of being at Mass General was part of the draw. "I thought this would be the perfect place to combine the basic science with the medical aspects of the research," she says. She flew to Boston to outline her hypothesis and ask Ausubel to take her on as a postdoctoral fellow, explaining her plan to use *Arabidopsis* to screen for *P. aeruginosa* virulence factors important in human disease. She was candid about the doubts of many other scientists.



"It was obviously a long shot, but why not?" Ausubel remembers thinking at the time. "I like to let people propose nnusual ideas and then pursue them. The work is always step-by-step, but at some point you have to take a leap." When Rahme joined his group in 1992, she was the only bona fide plant pathologist on the hospital grounds. Most of the medical staff had no idea that Ausubel's plant-genetics group was tucked away on the MGH campus. Infectious-disease expert Stephen Calderwood was an exception—a shared interest in how bacteria infect their hosts had already brought him together with Ausubel. He was intrigued with Rahme's screening idea from the outset, and has since collaborated with the plant experts to work on *P. aeruginosa* and other pathogens that cause problems for hospitalized patients.

One problem with bold ideas is that research money is generally inside, not outside, the proverbial box. Most federal funding decisions are made by committees of academic scientists, who tend to be steeped in the prevailing wisdom. When Rahme came along, that wisdom did not include using plants to study human disease. Just as Howard Goodman's vision and support enabled Ausubel to establish his lab, Ausubel gave Rahme the boost required to launch her search for virulence factors a decade later. She needed additional help to keep it going, of course, and noticed, serendipitously, an advertisement in the back of a scientific journal inviting applications for a fellowship to study *P. aeruginosa* infections and burns. The source was Shriners Hospital for Children. Rahme could look out the window of the Wellman Building and see the hospital. It sounded perfect.

She and Ausubel quickly set up a meeting with Ronald Tompkins at Shriners. They were disappointed to learn that the fellowship had already been awarded, but greatly encouraged by his enthusiasm for their proposal. Tompkins is a square-jawed, athletic-looking Southerner who could be a surgeon from central casting, and he inhabits an executive office with a panoramic view. But anyone who thinks that a conservative mindset is part of this picture would be wrong. He believed that pathogenesis researchers had been spinning their wheels since the late 1970s, when biochemical analysis had identified a group of bacterial targets that antibiotic drugs might hit.

Like Ausubel and Rahme, Tompkins is not afraid of taking risks. He had proved that a decade earlier, when he took time out from his surgical training at MGH to earn a doctorate in chemical engineering at MIT. He was an adult competing with whiz kids, and he says surviving that experience gave him the confidence to try pretty much anything that makes sense to him. Using simple hosts to screen for possible drug targets struck a chord, because Tompkins was already certain that science needed faster, simpler, more ethically acceptable ways to find additional chinks in bacteria's constantly evolving armor.

His own lab uses mice to study *P. aeruginosa*'s devastating role in burns, but there are many limitations to this model. If Tompkins wanted to pin down specific genes that make one strain of *P. aeruginosa* more virulent than another, for example, winnowing through all the candidate genes would require tens of thousands of mice. Given the ethical and practical barriers to such an experiment, "I would not embark on this lightly," Tompkins says. If plants could be used for the initial screening, thereby shortening the list of potentially important genes, far fewer animals would be needed. With this in mind, he arranged for a grant from the Shriners Burns Institute.

Rahme used for her initial study a collection of 75 strains of *P. aeruginosa*, originally developed at Berkeley, that included 30

Laurence Rahme, who is comfortable crossing boundaries, used plants to screen for virulence genes important in mammalian infection.

which had been isolated from human patients, 20 from soil, and 25 from plants. She screened these for isolates that caused the leaves of several types of *Arabidopsis* to rot and drop off. The most potent turned out to be PA14, a *P. aeruginosa* strain originally cultured from a patient. To confirm that this strain was equally virulent in lab animals, she tested it in Tompkins's mice and found that it killed more than three-quarters of the infected animals. Now the question was whether the bacterium used the same tools to attack both hosts, or whether it used a saw in one case and pliers in the other. To find out, Rahme induced mutations in PA14 that made it less virulent in plants, and then tested these mutants and the original bacterium in mice. What she found—that the strains less virulent in plants were also less virulent in mice—confirmed her original hypothesis. These results lent weight to the theory that at least some mechanisms of infection are so highly conserved that they bridge the plant and animal kingdoms.

These results were published in *Science* on June 30, 1995, with Rahme as the first author and Tompkins, Ausubel, and others sharing credit. Never before had anyone shown that a bacterium used the same virulence factors to infect a small leafy plant and a furry mammal. Yet the paper made few waves in the world of biology. "Most people disregarded it at the time," Ausubel said. Many researchers dismissed findings in *P. aeruginosa* because they saw it as an opportunist that did not need very good tools to invade hosts whose defenses were already weakened by burns, trauma, or diseases such as cystic fibrosis or AIDS.

Ausubel and Rahme, however, were eager to continue their research. If a scrawny little plant could be used to pinpoint *P. aeruginosa* virulence factors that play key roles in mammalian disease, could other simple hosts also be used to analyze pathogenicity? They began to envision a multi-host approach in which potential virulence genes would be screened in a series of models, with each host a bit more complex than the last on the great chain of being. They began to look for inexpensive, easy-to-mutate creatures with small genomes and short generation times that were more complex than plants and simpler than mice.

A graduate student in Ausubel's lab—Man-Wah Tan, Ph.D. '97, Jf '00, now an assistant professor of genetics at Stanford—found that when *P. aeruginosa* was fed to *Caenorhabditis elegans*, a tiny nematode popular with geneticists, it killed some or all of the worms in a petri plate. Further investigation showed that the mechanisms that killed nematodes were exactly the same as those the pathogen unleashed against plants and mice. As the team gained experience with *C. elegans*, Ausubel came to believe it was better than *Arabidopsis* for analyzing the intricate relationship between host and pathogen. And he wasn't sentimental about leaving behind the plant, even though he had made his career by using it. "No matter how good *Arabidopsis* is, in terms of ease of manipulation, *C. elegans* is easier than the plant as a system," he says. The nematodes have numerons advantages: colonies thrive on petri plates, they will eat any kind of bacterium smeared on the plate, and they don't need a greenhouse. Their three-day generation time, during which they speed from egg to egg-bearing adult, makes the life cycle of *Arabidopsis* seem positively sluggish. Finally, and very important to geneticists, the worms are hermaphroditic: because they don't need to breed with one another to produce young, hatchlings are genetically identical to the parent.

In the late 1990s, the quest to understand innate immunity was a major focus of pathogenesis research. Such immunity is the body's first line of defense, the part of the immune system that is built-in at birth, not acquired with age. "The major function of innate immune machinery is to recognize microbial pathogens and signal to the host that it may be under attack," Ausubel says. "Whether you're an *Arabidopsis* or a human, you have to recognize a bacterium and distinguish whether it is pathogenic or commensal" (part of the body's normal flora). Basic elements in innate immune responses include phagocytic cells that gobble up foreign materials, and chemicals that damage microbial invaders directly; beyond that, scientists are only beginning to identify the complex messaging systems an organism uses to recognize an attacker and formulate its response. In vertebrate animals, some of these signals activate a second type of defense: the acquired (or adaptive) immune system. This involves specialized B cells that generate antibodies, T cells that attack and kill invading pathogens, and cells that remember invaders and defend against them if they return.

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Pairs of *Arabidopsis thaliana* leaves demonstrate the effects of manipulating plant defense genes to enhance resistance to bacterial pathogens.

If scientists are going to pick apart the fine details of innate immunity, they need to be able to manipulate pathogen genes that control virulence and host genes that govern susceptibility. Rahme and Ausubel were expert at mutating *P. aeruginosa* genes related to virulence, but they were always looking for hosts that would make it easier to determine the impact of each of those individual mutations. Ausubel estimates that 4,800 genes may play a role in determining *P. aeruginosa* virulence, and that screening these in mice would require at least 48,000 animals, whereas the same genes could be screened using 4,800 petri plates of *C. elegans*, each one supporting about 50 of the near-microscopic nematodes. In this model, the virulence associated with a mutation is judged by seeing how long the worms live and how many progeny they produce. Neither of these outcomes is hard to measure.

By 1999 Ausubel and Rahme reported that they had used the nematode model to identify eight virulence-related factors in *P. aeruginosa*, five of which also determine how virulent a strain is in mice. Among these were two factors that had never before been identified—a finding that especially excited Stephen Calderwood, the MGH infectious-disease chief. The issue is not to recognize "archetypal pathogenesis genes," he says, because these are the long-standing targets of drugs that are fast losing their punch. "The trick is to find new kinds of pathogenesis genes, using a new paradigm, and that's what Fred and Laurie have proven works pretty well."

In their groundbreaking studies of infection, Ausubel and Rahme began by examining the pathogen's side of the story; the next task was to consider its victim. Just as some bacteria are fiercer than others, hosts also vary in susceptibility. Even when a severe infection is diagnosed correctly and antibiotics are started immediately, "patients survive or die during the first 24 hours based on their bodies' innate immune response," says Calderwood. Scientists are eager to figure out what makes the difference. Selectively mutating genes in a model host—whether a plant, a worm, or a mouse—is an obvious strategy. The genome sequence of *C. elegans* was determined in 1998, making it easier for researchers in Ausubel's lab to sift through the nematode's 14,000 genes, looking for those that make it more or less vulnerable to a pathogen of known virulence. Even the most easily killed worms die with eggs inside them, and their hatchlings can be used when a highly susceptible mutant is needed for an experiment.

So far, the most clinically significant finding is that nematodes can be used to identify virulence factors not only of *P. aeruginosa*, which is classified as a Gram-negative bacterium, but also of Gram-positive human bacterial pathogens. (Differences in the surface coatings of these two major categories of bacteria determine how they take up a special dye, called Gram stain. A model system that can be used to study both is more versatile in the laboratory.) Ausubel's team collaborated with Calderwood to identify two previously unknown virulence-related genes in *Enterococcus faecalis*, a Gram-positive organism that causes devastating infections, including septic shock, and often resists the hardest-hitting antibiotics. Drug designers may be able to use this knowledge to develop new drugs. The nematode model is also being used to identify virulence factors of other Gram-positive bacteria, including members of the *Salmonella*, *Streptococcus*, and *Staphylococcus* genera.

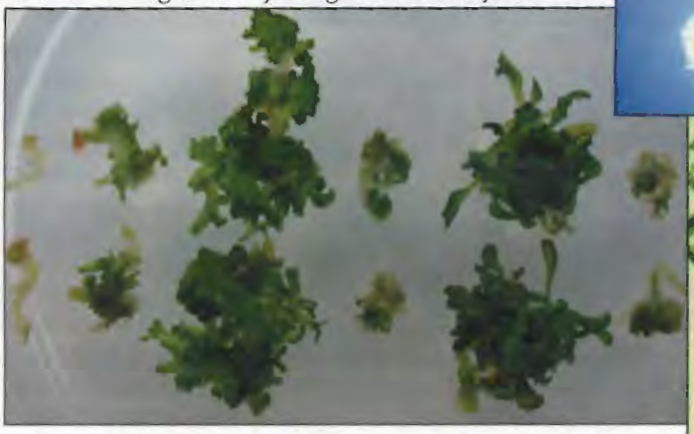
Even when antibiotics are started immediately, "patients survive or die during the first 24 hours based on their innate immune response."

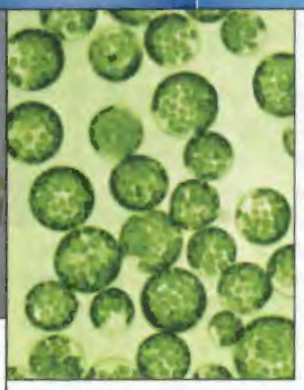
While the Ausubel lab focuses on nematodes, Laurence Rahme's team is busy with *Drosophila melanogaster*, the fruit fly that has been used in genetics laboratories for more than a century. Of the 300 or so genes known to cause human diseases such as muscular dystrophy and colon cancer, about two-thirds have corresponding genes in the fly. With this track record, the humble fly is recognized as one of the best of all animal models. Rahme had read reports from several groups about the fascinating and versatile *toll* (*wild* or *mad* in German) gene, which encodes the Toll receptor on the surface of fly cells. This receptor is a sensor that detects fungal pathogens and sets off a chain of events that musters a protective response. When the gene is mutated, the fly's innate immune system can't successfully fight off infection. Moreover, other investigators had shown that human cells have Toll-like receptors, which also sense warlike pathogens and sound alarms that call antibody-producing B cells and cell-killing T cells into battle.

But the conventional wisdom was that where bacteria are concerned, Toll receptors in flies and Toll-like receptors in more com-

plex animals are sensitive only to Gram-positive types such as *Enterococcus* and *Staphylococcus*. Rahme wasn't willing to take that at face value. Experiments in her lab soon demonstrated that PA14 and other highly virulent strains of Gram-negative *P. aeruginosa* also used this route to kill flies, just as they wiped out *Arabidopsis* and mice. Her lab now uses plants, flies, and mice as model systems. Rhame and her colleagues hope that pairing *Drosophila* and mice as screening systems will be particularly fruitful because both models have been extensively studied, their genomes are sequenced, and it should be easier to locate susceptibility genes that are players in both species.

In terms of potential drug development, one of the most interesting genes Rahme's lab has found is a transcription regulator gene they call *mvfR* ("multiple virulence factor Regulator"). Transcription regulators take signals from the cell surface and initiate a series of events that tell the pathogen what to do next. When bacteria colonize a wound or other site of infection, such as the human gut, they talk among themselves. When the right time comes, *mvfR* tells virulence-related genes that it's time to churn out their proteins. But when the bacterial colony reaches a certain density, *mvfR* senses that it's time to call off the attack and signals the virulence genes to shut down. Rahme's team is working feverishly to figure out exactly how this works, with the ultimate goal of discovering drugs that could send the "retreat" signal at the first sign of infection, before virulence proteins have ravaged the host. Compounds that could call off bacterial attack may already be archived in chemical libraries that exist at Harvard and pharmaceutical companies—or it may be possible to design a novel drug from scratch. Rahme doesn't know yet. But it's obvious that her ideas, once considered somewhere in left field, have moved considerably closer to the mainstream.





Upper right: Jen Sheen manipulated protoplasts (cells stripped of their outer layer) to form diverse cell and tissue types. Lower right: *Arabidopsis* protoplasts. Above: Shoot and leaf formation activated by the genetic signaling pathway in an experimental tissue culture.

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WHILE AUSUBEL AND RAHME have branched out from plants, which were their first love, molecular geneticist Jen Sheen, Ph.D. '86, has remained happily in "the green world" for two decades. Relying entirely on plant models, she has made extraordinary discoveries about how the innate immune system reacts to a bacterial threat. Sheen's love of plants and learning dates to her childhood in Taiwan, where she was strongly influenced by her schoolteacher parents: her mother cultivated orchids and her father was passionate about his garden. Sheen knew early on that she wanted to be a biologist, but in high school she was torn between botany and zoology. Although savvy adults told her that animal researchers have brighter career prospects, and although she believes that using animals to understand human disease is ethical, she knew that biologists who use animal models eventually have to kill some of their research subjects. Says Sheen, "I read something once about the difference between the scientists who study plants and animals, and it said that the people who study plants really love animals. And that's true of me."

Sheen explored signal transduction, the umbrella term for chains of events that ferry information from the environment into the cell.

Sheen moved to Mass General because Howard Goodman and Fred Ausubel invited her. In the 1980s, when identifying and cloning a single gene took months, or sometimes years, Sheen cloned 30 genes and published eight papers while earning her Ph.D. in cellular and developmental biology at Harvard. These are crucial photosynthesis genes from maize, an important crop plant, and she hoped that identifying them would help increase food yields and fuel the green revolution. On the strength of this work, Sheen wanted to skip the initiation rite of being a postdoctoral fellow in someone else's lab, and instead tackle the development of a new model system. Engineering genes into plants was a slow business, and she wanted to devise a way of speeding it up by doing experiments in cells, instead of whole plants.

But no such system existed, and many people assumed it could not be done. Goodman and Ausubel were more optimistic. The MGH molecular genetics department's large endowment from Hoechst made it possible for Sheen to set up her own lab in 1987. "They basically said 'Do whatever you want,' and nobody came in and said 'Don't do this, don't do that,'" Sheen recalls. "If you apply for grants, you cannot do anything risky. There was no way I could have done adventurous research without coming here."

She went to work immediately on the faster, cheaper experimental system she had dreamed of. The key turned out to be using protoplasts, plant cells that have been stripped of their outer layer but can be maintained in a laboratory dish. Although these cells are physically naked, they still have receptors that sense what's

happening around them and that trigger complex responses inside the cell, much as cells in a growing plant would do. Drop a new gene into the dish and only 3 to 6 hours are needed to see if the cells have taken it up, compared with 24 hours for the same experiment in *Escherichia coli* (a popular model in genetics labs), two days for cultured mammalian cells, or as long as several weeks in whole plants.

With the protoplast system in hand, Sheen set out to explore the genetic control of innate immunity, focusing especially on signal transduction. This is the umbrella term for chains of events that ferry information from the environment into the cell, where it is processed, integrated with other signals, and responded to. In 1990, a self-described "late converter," Sheen began using *Arabidopsis* to confirm what she had uncovered in protoplasts. Compared with animals, which can summon antibodies and T cells to fight off attackers, plants seem to have relatively few defenses in a dangerous world. "They have to protect themselves from constant assault and they can't run away," she says, "So I think they probably have an immune response in every cell."

Jen Sheen, a molecular geneticist, has unraveled mysteries of the immune system.

When a pathogenic bacterium menaces a living creature, the first step in the host's innate immune response is to recognize signature proteins that identify the microbe as dangerous. Toll receptors do this job in *Drosophila*, and their counterparts—Toll-like receptors—do the same in human beings and animals. Recognition triggers an orderly cascade of events that carries signals from the cell's outer wall to its interior as efficiently as microwave towers relaying a telephone call from Boston to Los Angeles.

It turns out that plants are equipped with their own version of these cell-surface receptors. Signals picked up by these sentries are thought to travel next via enzymes known as MAP (mitogen-activated protein) kinases. Although numerous MAP kinases have been described in simple and complex animals, the hard part has been determining where each one stands in relation to the others. This is like knowing that a Boston-to-Los Angeles phone call will pass through Pittsburgh, St. Louis, and Amarillo, but not knowing which one comes first. The challenge is to arrange each step on a map inside the cell.

Sheen went after this problem several years ago, armed with the *Arabidopsis* genome sequence, sophisticated software for identifying possible MAP kinase genes, and considerable expertise with protoplast and plant models. She also had a $4.5-million, five-year grant from the National Science Foundation's plant genome program, a vegetable version of the much better known Human Genome Project. In February 2002, the first publication from her project made a splash when it appeared on the cover of the prestigious journal *Nature*.

The article described an elaborate chain of events that is touched off when flagellin, a highly conserved bit of protein that is a component of bacterial filaments, binds to a surface receptor. Information from the receptor is passed by specialized chemical "elicitors" to a chain of MAP kinases that fall into place like a row of dominoes given a push. Steps in this cascade were identified first in protoplasts and then verified in *Arabidopsis*, and this was the most complete description of such a linkage published so far. Subsequent plant experiments showed that the MAP kinase cascade is triggered by fungal as well as bacterial sensors on the cell surface, suggesting that information from various surface receptors shares pathways inside the cell, just as

phone calls originating in Boston and New York will travel some of the same wires en route to San Francisco.

The research team, which included Sheen, Ausubel, and members of their labs, compared their plant findings with less-complete descriptions of MAP kinase cascades in other creatures, including *Drosophila*, nematodes, and mammals. It's not clear yet whether a particular MAP kinase from the plant can be mapped to an exact equivalent in mammals, but Ausubel says, "We can do that in *C. elegans*." There's also a good match between what happens in plants and in flies. These findings are of considerable interest not only to biomedical researchers, but also to evolutionary biologists (see sidebar). "If innate immunity is ancient and conserved," Ausubel says, "then pieces of the machinery will be the same no matter what organism you look at. I think we might have found one of those pieces in the MAP kinase signaling cascade."

In addition to shedding light on similarities that originated millions of years ago, this work is expected to have medical implications for the future. Sheen believes that the systematic analysis of MAP kinase signaling in plants will help researchers recognize equivalent mechanisms in the human genome. Her hunch is that signaling events initiated by bacteria-sensing receptors will turn out to be important in causing life-threatening sepsis, and that a drug may someday be able to step in and keep the wrong dominoes from falling.

IN LITTLE MORE than a decade, the idea of using plants and other simple hosts to explore the complexity of human disease has moved from the margins toward the mainstream of science. One early sign of respectability among academic scientists was a 1999 National Academy of Science colloquium on host-pathogen interactions in plants and animals, where Rahme was invited to speak. In mid 2000, the American Society for Microbiology's annual meeting included a session on this topic for the first time. Equally telling is who pays for the research. The Shriners Burns Institute got on board early, first underwriting studies of *P. aeruginosa's* trans-kingdom virulence strategies back in 1993. The National Institutes of Health has since helped support work in the Ausubel and Rahme labs, and Aventis has invested $4.1 million in studies using the multi-host screening system to identify "novel anti-infective targets."

The possibility of someday being able to prescribe anti-infective drugs is very exciting to an MGH physician such as Stephen Calderwood. Antibiotic resistance arises because traditional antibiotics kill the most vulnerable bacteria in a population, leaving the toughest survivors to pass along their genes. Calderwood envisions anti-infective drugs that would block the surface structures bacteria use to attach themselves to host cells, leaving the microbes alive but disarmed and harmless. Such a strategy would avoid the selection pressure that gives rise to resistant strains. "Any drug that did this would be a breakthrough," he says.

Both Calderwood and Ronald Tompkins see great promise in the work of pioneers like Ausubel, Rahme, and Sheen. As the problem of antibiotic resistance worsens, "We need to be in discovery mode," Tompkins says. He sees multi-host systems as the best way to identify what he calls "silent but deadly" pathogenesis genes. Calderwood believes that *C. elegans* will eventually be used to assess possible anti-infective compounds as well. Radical new therapeutics won't be identified overnight, of course, and developing such drugs will most likely require the expertise and financial resources of major pharmaceutical companies, which recognize that basic research being done at MGH today may yield insights the companies can convert into tomorrow's products.

Meanwhile, the pioneers of plants and simple hosts go largely unnoticed in the crowded corridors of Mass General. It's not that they are prophets without honor in their own country, exactly. It's just that most physicians and patients have no idea what these researchers are up to and how important their work could turn out to be. This unique subculture of researchers "needs to have some prominent successes," Tompkins says, "and then people will know who they are." ∇

LEPIDOPTERIST Naomi Pierce, Hessel professor of biology in the Faculty of Arts and Sciences (see "A Life with Lycaenids," July-August 2001, page 42), has joined Frederick M. Ausubel, professor of genetics at Harvard Medical School, in investigating whether a plant can simultaneously fight a microbial infection and a plant-eating predator. At the biochemical level, plants use the salicylic-acid pathway to battle fungal and bacterial pathogens, and the jasmonic-acid path to discourage insects from eating them. "What's the cross talk between those pathways?" Pierce asks. "If you are making lots of salicylic acid, will it be harder to suddenly increase secretions of jasmonic acid if an insect comes along?"

She and Ausubel infected *Arabidopsis* with *Pseudomonas syringae*, then introduced larvae of the cabbage looper moth, little green inch worms, to see what happened. Although the experiments are ongoing, Pierce says one thing seems clear: the ability of the plant to fend off the herbivore depends on which bacterial strain infects it. If the strain is not recognized by plant-resistance genes, the plant is open to bacterial infection and more vulnerable than usual to the predations of the inchworm. But if the strain is recognized by plant resistance genes, then the plant is able to confine bacterial damage to local areas *and* to fend off the cabbage looper.

The researchers don't know if this type of bacterium acts like a vaccine and "primes" the immune system, or if cross talk between the salicylic- and jasmonic-acid pathways enables the plant to defend itself on two fronts at once. These signaling pathways are widely used, however, so whatever emerges from this research could have broad implications. The advantage of simple models like *Arabidopsis*, Pierce says, is that they "enable you to get a handle on bigger processes that you might never have guessed existed."

The realization that pathogens can use the same virulence strategies to invade plants and people, and that MAP kinase cascades may be a common feature of self defense, comes as no shock to Pierce. "Proteins are built by amino acids in all creatures, so maybe it's not surprising that pathways are similar across disparate groups of organisms."

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