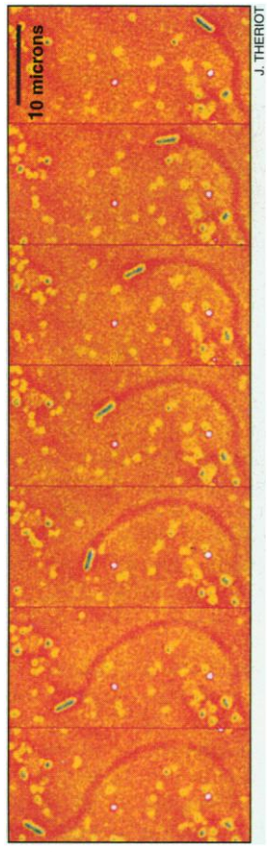
CELL BIOLOGY

Cell Movement Tale Told by Bacterial Tail Protein

Cells can creep. In embryos, cells slowly crawl to specialized destinations in the body. In cancer patients, tumor cells spread by utilizing a structural protein, actin, to push sheetlike appendages out of their membranes. But just how this happens is "one of the great unsolved problems of cell biology," according to Julie Theriot, a cell biologist at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts.

A key to solving this puzzle seems hidden in bacteria. These simpler cells also use actin for motion—but in a radically different way. Certain microbes that cause dysentery and meningitis take up actin molecules from the cells they've infected and deposit those molecules immediately to the rear, forming a rigid tail to push against. By pushing against each newly built section, the bacteria can gather sufficient force to punch through the cell wall and infect a neighbor.

Now Theriot and a colleague have pinned down a bacterial protein responsible for building these actin "tails." That's a breakthrough in itself. What's more, in doing so, they have also developed a model for investigating their higher cell mystery. And, in a biological double bonus, the same protein may hold the key to developing a vaccine strategy that can be used against dysentery.



Tell-tail. *E. coli* don't normally move with a tail. But equipped with a tail-producing gene from another species, this mutant microbe made a tail and began squirming about.

In last week's *Proceedings of the National Academy of Sciences*, Theriot and infectious-disease specialist Marcia Goldberg of Albert Einstein College of Medicine in New York report taking a gene from one of these infectious bacteria, *Shigella flexneri*, placing it in tailless *Escherichia coli*, and watching as this microbe promptly grew an actin tail and began moving about. The work indicates that the protein produced by the gene, IcsA, may be the sole *Shigella* protein required for its actin-based movement.

That mutant tail has a lot of potential, say other researchers. Timothy Mitchison, a cell biologist at the University of California, San Francisco, says, "IcsA was known to be necessary for movement, but this is the first test showing that it's sufficient. That's very important." The importance derives from the fact that researchers can now use IcsA in simple organisms to study sheet formation in sophisticated cells. Proteins similar to IcsA may be at work in these sheetlike structures, but the higher cell membranes make the activity hard to spot. "The back end of a *Shigella* bacterium mimics some activated state of the plasma membrane [in moving cells] which we don't yet understand," explains Mitchison. With a simple model, that understanding may be creeping a bit closer.

The discovery, 6 years ago, that *S. flexneri* missing the IscA gene couldn't move, made by bacteriologist Philippe Sansonetti and colleagues at the Institut Pasteur in Paris and a Japanese team led by Chihiro Sasakawa at the University of Tokyo, was the first hard evidence that the IcsA protein is involved in actin-based movement. But the finding left scientists with two questions. First, what exactly did the protein do to actin to produce the tails? And second, was IcsA capable of producing the tails on its own, or did it need help from other proteins?

Sansonetti's team started to answer the first question in 1992. Using an electron microscope to take stop-action images of bacteria and a stained version of the protein, they showed that IcsA and actin monomers—protein subunits floating free in the cytoplasm—accumulate at one pole of the bacterium, where monomers drop into place, forming a longer molecule the bacterium can push against. The actual movements may depend in part on the bacterium's natural Brownian motion: Each forward vibration creates space at the rear pole for new monomers, which are drawn from the cell cytoplasm and snapped into place. The bacterium's ability to jiggle backward again is blocked—and the result of many such vibrations is forward motion (*Science*, 17 March, p. 1593).

At the same time, Theriot and Daniel Portnoy, a microbiologist at the University of Pennsylvania, had gleaned much the same information about another bacterium that uses actin tails to move, *Listeria monocytogenes* [(*Nature* **357**, 257 (1992)]. And French and German scientists found an analog to IcsA in *L. monocytogenes*, a protein called ActA. Like *S. flexneri* missing the *icsA* gene, bacteria lacking the gene for ActA fail to form actin tails.

But whether IcsA and ActA, when present, could make tails by themselves was still undetermined. After Goldberg, who was working with Sansonetti at the time, met Theriot at a conference in 1992, the two agreed on the best way to determine the extent of IcsA's role: to force *E. coli*, a bacteria that normally uses flagella to move and does not have the *icsA* gene, to make the protein, and see whether a tail developed.

Goldberg put the *icsA* gene into an *E. coli* strain lacking an enzyme that normally degrades foreign proteins such as IcsA. She found the protein promptly expressed on the microbes' surface, concentrated near one end of the organism. Last fall, she sent the hybrid strain to Theriot, whose laboratory owns a powerful microscope equipped with an ultraviolet light source and video and still cameras. Within minutes of placing the *E. coli* in an extract of cytoplasmic fluid, Theriot found the bacteria formed actin tails and began zipping about.

And these souped-up bacteria were just as fast as *S. flexneri* in cultured cells, achieving the highly competitive rate of almost 1 millimeter per hour. (The findings do not strictly rule out the possibility that another unidentified protein common to both *S. flexneri* and *E. coli* assisted in this feat, but the researchers hope to eliminate this prospect soon by testing whether IcsA-coated synthetic beads attract actin.)

Such molecular-level insights into the way *S. flexneri* and *L. monocytogenes* form actin tails may sharpen researchers' view of motility in higher organisms. Biologists have known for decades that tumor cells, white blood cells, and most cells in the embryos of developing organisms polymerize actin to form the sheetlike extensions, or "lamellipodia," that allow them to ooze slowly from place to place. Because this process is obscured by the complexity of the eukaryotic cell membrane, however, its exact biochemistry remains hidden as well.

The process is easier to observe in the mutant *E. coli*, because actin-based motility in bacteria represents what Theriot calls "a stripped-down, Ferrari race car version of what we think is going on in lamellipodial motility." Sansonetti, who started much of this research, is cautious but optimistic: "It's hard to know how much this system will tell us about

the real physiology of eukaryotic cells ... but this aspect of motility is really quite exciting."

While that mystery is being investigated, what's already known about IcsA's role in bacteria could open the way for a *Shigella* vaccine. And that could have important public health implications, because conventional antibiotic remedies against S. *flexneri* and its cousin S. *dysenteriae*—which cause diarrheal epidemics resulting in hundreds of thousands of deaths every year among infants and children in developing countries—are faltering.

Last year Sansonetti sent motionless, tailless S. *flexneri* strains lacking the *icsA* gene to Thomas L. Hale, chief of the Department of Enteric Infections at the Walter Reed Army Institute of Research in Washington, D.C. Hale deleted another gene—one that regulates the bacteria's ability to take up the nutrient iron, thus shortening its lifetime—and recently completed studies showing that this hybrid strain's infectious ability is crippled: It doesn't cause dysentery in rhesus macaque monkeys.

On 11 July, he will begin administering the whole bacteria to 30 human volunteers to find the minimum safe dose of bacteria necessary to evoke an immune response. After pinpointing the safe dose, Hale says, he'll conduct more safety studies in a larger group of volunteers, and then progress sometime next year to "challenge studies" testing vaccinated volunteers' immunity to the unattenuated microbe. "In southern Asia, there are strains of *Shigella* resistant to almost all of the antibiotics normally used against enteric organisms," says Hale. Vaccines based on IcsA mutants "are probably one of the best alternative approaches" to controlling the disease, he says.

Karen Kotloff, an infectious-disease specialist at the University of Maryland's Center for Vaccine Development in Baltimore, says Hale's trials are "very well advised." The *icsA* deletion alone probably would not attenuate *Shigella* sufficiently to make it safe at immunogenic doses, she says, so "using two mutations—one that shortens *Shigella*'s life and another that contains the infection—is a very reasonable approach."

Theriot notes that S. *flexneri* are already "much better cell biologists than humans are." If Hale's approach bears fruit, they may turn out to be better immunologists as well.

–Wade Roush

BIODIVERSITY RESEARCH

Fifty Shades of Rain-Forest Green

On a schoolchild's map of the world, the oceans are colored blue, the Amazon rain forest is green, and the sands of the Sahara are tan. Biologist Hanna Tuomisto of the University of Turku in Finland thinks this kind of child's-paint-box scheme has hampered creative thinking about the Amazon basin for years, as many researchers treated all 4 million square kilometers of the rain forest as a homogeneous block.

On page 63, Tuomisto and colleagues present an attempt to change such monolithic views, using new data from satellite images and laborious field surveys. Their results reveal that maps of the Peruvian Amazon ought to be colored in not one but perhaps 50 shades of green to indicate a patchwork of different forest types. "They're addressing the conventional view of the rain forest as homogeneous, as the green hell on a map," says Paul Colinvaux, a tropical ecologist at the Smithsonian Tropical Research Institute in Panama. "We very badly needed this kind of data." This new picture of the Amazon also has implications for some key ecological issues: How did so many species arise in the rain forests—and how can they be saved now?

Tuomisto and an interdisciplinary team from Finland and Peru explored the Peruvian Amazon at scales ranging from a meter to hundreds of kilometers. For the small-scale studies, they spent five field seasons in tent camps, walking transects and sampling soils, ferns, shrubs, and trees. They found that the mix of species varied according to soil fertility and topography. Valleys had more diversity than hills, for example, and forests growing on different soils had different species compositions.

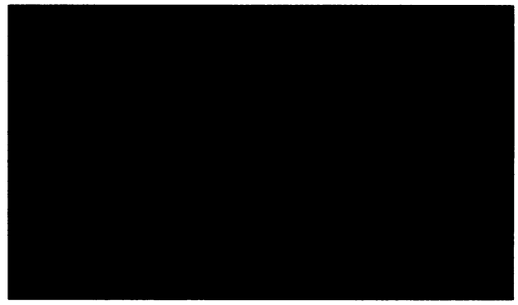
This close-up view was buttressed by the team's analysis of Landsat images, in which false color patterns are created by physical properties of soil and vegetation. If the forest were homogeneous, each pixel in a satellite image would be the same color as its neighbors, explains Tuomisto. Instead, the team saw a mosaic of colors worthy of a Seurat painting. On average, a 30-kilometer transect contained four colors or habitats. In a single 34,000-square-kilometer image, as many as 54 distinct habitats were visible. All this means that the Amazon forest is more heterogeneous than it looks, says Tuomisto.

To be fair, other ecologists who have scrambled their way through the jungle, especially in Southeast Asia, know that rain forests aren't homogeneous, says forest ecologist Stephen Hubbell of Princeton University. But he says the new work offers a much-needed method to survey large-scale variability. "We need to pay much more attention to the way diversity is arranged on landscapes, and so we need to scale up. And that's just what they've done," he says.

The work also has implications for the enduring mystery of how tropical rain forests acquired their remarkable biological diversity. "The majority opinion has been that the rain forests have high species diversity despite very little physical variation," explains Tuomisto. So biologists have postulated that today's forests contain species-rich areas that are relics of the ice ages, when the climate was drier and forests shrank into small refuges. According to this theory, those refugia became centers of speciation thousands of years ago and retain great diversity today.

The new study suggests a simpler explanation: Diversity of habitat, created by physical factors, led to the wealth of species. "The problem has been, if the Amazon is one huge forest, how do you get such disjunct distributions," says Colinvaux. "And now they're saying that it's not one huge forest; it's heterogeneous. This makes the refugium hypothesis unnecessary."

But the new data don't refute that hypothesis, counters Hubbell. In fact, he says, the two ideas are not mutually exclusive, because Ice Age soils presumably varied too. Tuomisto agrees, saying it's premature to chuck out any theories. The next step is to verify and define the physical factors behind the Landsat color patterns. The team suspects topography and soils play a role—but they'll need a massive field effort to prove it.



H. TUOMISTO

Mixed greens. On this Peruvian hill some tree canopies are densely packed, while others stand out individually.

"This is the tip of the iceberg. It will take lots more ground-truthing to refine the method," says Hubbell.

A highly heterogeneous forest also supports a new philosophy in conservation biology: Diversity of habitats, as well as sheer numbers of species, should be considered when creating biodiversity preserves. "You should get a reasonable sample of all the habitats into the conservation program, even if some habitats have relatively fewer species," says Tuomisto. Debate on such issues is likely to continue. But it's already clear that would-be artists painting Amazonia will have to expand their palettes beyond a single shade of green.

–Elizabeth Culotta