One of cell biology's first mysteries is coming under renewed scrutiny as new techniques allow researchers to follow in cells' steps

Cell Migration Research Is on the Move

Anton van Leeuwenhoek was fascinated by the movements of the tiny creatures he discovered in rainwater in 1675. In a letter published by the *Philosophical Transactions of the Royal Society of London*, he described living atoms that put forth little horns, extended and contracted, and had pleasing and nimble motions.

Cell-sized movements still draw the attention of biologists today. "The cool thing about cell motility is it's one of the first research programs in [cell] biology," says Thomas Stossel of Brigham and Women's Hospital and Harvard Medical School in Boston. No less appealing is its central involvement in a wealth of biological processes. Migrating cells shape organs and tissues in developing embryos, hunt down infectious agents, generate new blood vessels, and close wounds. And cell migration gone awry lies at the root of many diseases, including metastatic cancer, arthritis, and neurological birth defects.

Yet cell migration has not enjoyed the popularity of some of its sister fields. "The irony is that cell migration predates by hundreds of years the molecular biology revolution, yet it has been somewhat of a sideshow," says Stossel. Part of the problem is that cell migration has been a fractured discipline, composed of many subfields. "There are so many interacting pathways and processes that it's very difficult for any one lab to work on all of them," says Alan Hall of University College London. "It's kind of mind-boggling to try and put it all together."

But powerful new tools are beginning to help assemble a more unified picture of cell migration. Stripped-down model systems and methods to search for migration-related genes and proteins on a large scale are enabling researchers to identify the multiplicity of players and track their interactions. Techniques to monitor and manipulate mechanical forces in living cells are providing clearer views of the dynamics of cell movement. And computer models are helping researchers design experiments, test hypotheses, and integrate the wealth of data.

"Finally, cell migration is starting to emerge as an integrated, whole field," says Rick Horwitz of the University of Virginia in Charlottesville. Indeed, in September 2001, the National Institute of General Medical Sciences (NIGMS) in Bethesda, Maryland, awarded an $8 million "glue" grant to the Cell Migration Consortium, a multidisciplinary, multisite group led by Horwitz and Thomas Parsons, also at the University of Virginia. Over the next 5 years, NIGMS expects to award $38 million to this diverse group of biologists, chemists, physicists, and computer scientists, who plan to develop new technologies and approaches for tackling cell motility.

Poetry in motion. Van Leeuwenhoek's microscope (*inset*) revealed living atoms' pleasing and nimble motions.

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The crawl

Modern theories of cell migration can be traced to the early 1970s, when Michael Abercrombie of University College London published a series of five articles in *Experimental Cell Research*. Based on studies using video and electron microscopy of cells crawling on solid surfaces, Abercrombie suggested that cells move by extending protrusions and forming new contacts with the surface. The cell body is then drawn up to the new adhesions by a "system of contractile filaments."

This largely mirrors the current understanding of cell migration. Most researchers agree that eukaryotic cells extend protrusions to push their membranes outward by harnessing the growth of filaments of a protein called actin. Cells then attach their protrusions to the extracellular matrix, a mesh of proteins that surrounds cells and holds tissues together. To grab hold, cells use integrins, proteins that span the cell membrane and recruit signaling and structural proteins, including actin fibers, to form adhesions. Adhesions serve as points of traction over which the cell can pull the bulk of its body forward. Proteins that tug on actin filaments to generate force do the towing; they're called myosins and are similar to proteins that allow muscles to contract. Finally, the cell releases its rear attachments, marking the end of a full cycle of motion.

A problem of numbers

Although this general description appears simple, it belies a complicated and often mysterious set of processes. Each step involves many players, and "there are a lot of subprocesses, each of which involves scores of molecules," says Horwitz. One of the main goals of cell motility research is to identify these supporting

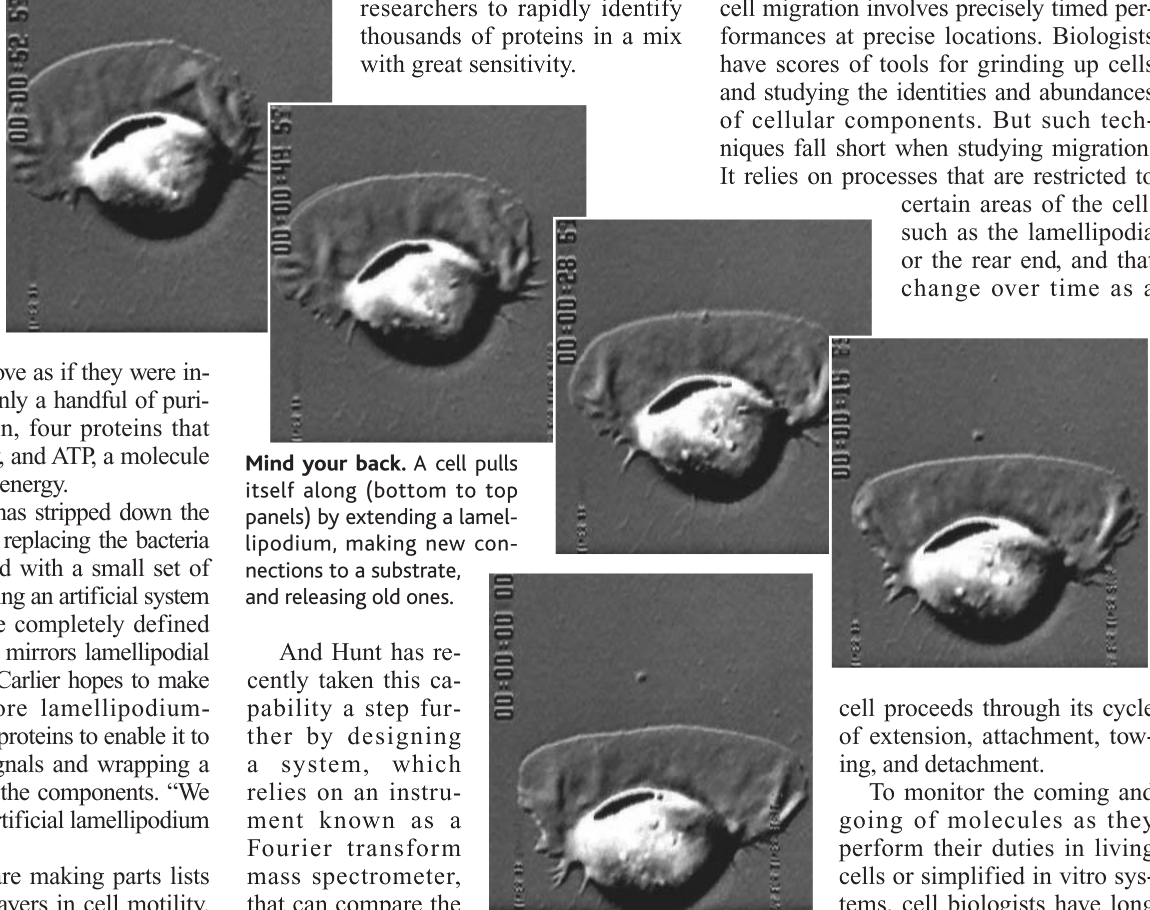
actors and determine their function and relative importance.

Some researchers are tackling this multiplicity by developing models that strip away at least part of the complexity. Marie-France Carlier of the National Center for Scientific Research in Gif-sur-Yvette, France, for example, has assembled an actin-based moving machine in a dish that recreates many of the characteristics of how crawling cells extend protrusions called lamellipodia. Carlier monitors the ability of the components to produce movement by watching bacteria called *Listeria* and *Shigella*. These microbes infect cells and enlist several of the components that cells normally use to extend lamellipodia to power the bacteria's own travels within the host's cytoplasm. In experiments reported in *Nature* in 1999, Carlier succeeded in making the bacteria move as if they were inside a cell by adding only a handful of purified components: actin, four proteins that regulate actin assembly, and ATP, a molecule cells use as a source of energy.

More recently, she has stripped down the system even further by replacing the bacteria with latex beads coated with a small set of known proteins, producing an artificial system whose components are completely defined and that in many ways mirrors lamellipodial protrusion. Ultimately, Carlier hopes to make her system even more lamellipodium-like, adding regulatory proteins to enable it to respond to external signals and wrapping a cell membrane around the components. "We are trying to make an artificial lamellipodium from scratch," she says.

Other researchers are making parts lists to track the various players in cell motility. "The idea is to produce an accounting of the proteins and their modifications at defined places in the cell," says Mark Ginsberg of the Scripps Research Institute in La Jolla, California. His team has constructed baits with which to fish out proteins that interact with the portions of integrins that reside within cells. Because integrins are composed of two protein subunits, the researchers make their baits by using small protein modules to splice together fragments of the two subunits and mimic integrins' configuration inside cells. They then attach the engineered proteins to beads and expose them to cellular extracts. Proteins that normally interact with integrins stick to the beads and can be analyzed. "We've been doing this with success," says Ginsberg, who first described the technique in 1998. But until recently, researchers have been limited to tracking the binding of a few proteins at a time.

Now new techniques are poised to help Ginsberg and others searching for interacting protein partners scale up their searches dramatically. "Mass spectrometry is probably the ideal way of getting at this," says Donald Hunt of the University of Virginia. Hunt has designed mass spectrometers—instruments that can determine the molecular weights and amino acid sequences of protein fragments—that enable researchers to rapidly identify thousands of proteins in a mix with great sensitivity.



Mind your back. A cell pulls itself along (bottom to top panels) by extending a lamellipodium, making new connections to a substrate, and releasing old ones.

CREDITS: M.-F. CARLIER *ET AL.*

And Hunt has recently taken this capability a step further by designing a system, which relies on an instrument known as a Fourier transform mass spectrometer, that can compare the protein content of two different mixtures. It can pick out proteins that are differentially expressed in two types of cells, or in the same types of cells under different conditions—say, proteins present in mobile cells but not sedentary cells—and selectively identify them.

Researchers are also casting genetic nets to do large-scale searches for genes that participate in cell migration. A team led by Joan Brugge at Harvard, for example, is screening genes expressed by highly mobile cells derived from invasive breast tumors for their ability to boost the mobility of normal breast cells. So far, the researchers have fingered two genes coding for proteins that, although previously identified, were not known to play a role in migration. And using a new vector for delivering genes into cells, developed by George Daley of the Massachusetts Institute of Technology, Brugge thinks her search is about to speed up. The vector enables researchers to insert genes into cells efficiently and then retrieve them easily—making it possible to sequence the genes quickly once they've proven their abilities, in this case, by facilitating migration. "This will just save months and months of work," Brugge says.

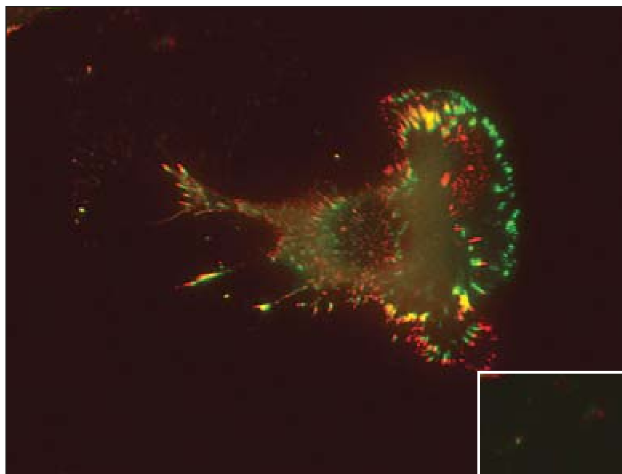
The right place at the right time

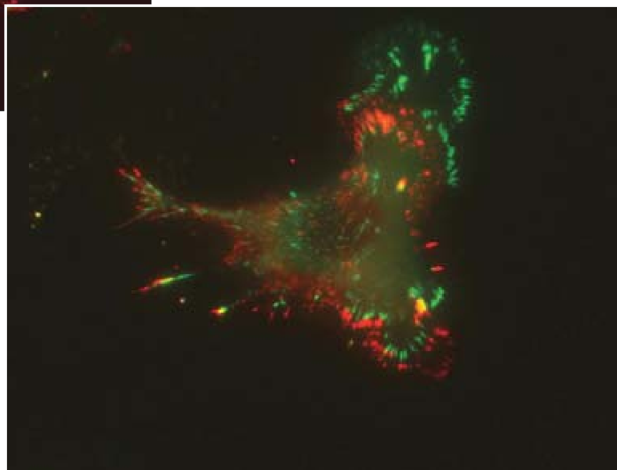
Besides having a large cast of characters, cell migration involves precisely timed performances at precise locations. Biologists have scores of tools for grinding up cells and studying the identities and abundances of cellular components. But such techniques fall short when studying migration. It relies on processes that are restricted to certain areas of the cell, such as the lamellipodia or the rear end, and that change over time as a cell proceeds through its cycle of extension, attachment, towing, and detachment.

To monitor the coming and going of molecules as they perform their duties in living cells or simplified in vitro systems, cell biologists have long relied on fluorescent labels. By following labeled actin monomers under the microscope, for example, researchers have witnessed the polymerization and depolymerization of actin filaments in real time. But in the last 2 years, new advances have greatly expanded the capabilities of fluorescence microscopy, enabling researchers to get a better view of when and where proteins involved in cell migration do their jobs.

Advances in technologies to track single molecules, for example, allowed Kurt Amann, working in Thomas Pollard's lab at Yale University, to watch how a protein complex found in lamellipodia, called the Arp2/3 complex, regulates actin branching. In work published in the December

2001 issue of the *Proceedings of the National Academy of Sciences*, Amann used a technique called total internal reflection fluorescence microscopy (TIRFM) to follow the growth of isolated actin filaments in the presence of the Arp2/3 complex. Like many before him, Amann used fluorescent actin monomers to track growth. But by using TIRFM, he was able to monitor single actin filaments—a trick normally beyond the power of traditional microscopy. To avoid noise from other labeled monomers, TIRFM shines a laser beam on the specimen at an angle that is completely reflected by the interface between the glass slide that holds the specimen and the solution in which the specimen is immersed. In this way, only fluorescent molecules that are within 100 nanometers of the interface are excited.





Tracking adhesions. In both images, adhesion proteins called paxillins move from their initial positions (first timepoint in red) as the cell crawls (second time point in green).

Amann's TIRFM observations shed light on how the Arp2/3 complex assembles actin branches—an important issue because branched networks are thought to provide a sturdy scaffold to push the cell membrane forward during extension. "He found that activated Arp2/3 complex forms branches at random sites along the length of actin filaments," says Pollard. But, cautions Carlier, "the [TIRFM] technique is not devoid of artifacts." Branches that bump into the nearby glass, for example, probably don't grow normally. Based on other studies, Carlier and others think Arp2/3 complexes form branches from the ends of actin filaments, not their sides. Despite numerous experiments addressing this controversy, including two structural studies of the Arp2/3 complex (*Science,* 28 September 2001, p. 2456, and 23 November 2001, p. 1679), the question remains unresolved.

To track filaments in living cells, researchers such as Clare Waterman-Storer at Scripps have opted for loading cells with very low amounts of fluorescent monomers to reduce background noise. This strategy has the added advantage of creating irregularly labeled, or "speckled," filaments that have a distinctive pattern and can thus be followed under a microscope as they move within the cell.

Now Waterman-Storer is trying to inject new power into the technique by collaborating with computer scientists and engineers to automate speckle tracking. Even trained experts find it hard to accurately measure filament movement, assembly, and disassembly by following speckles with their eyes. With computers, however, it should be easier to monitor the direction and magnitude of movement and to extract a greater fraction of the data generated by each experiment. Says Waterman-Storer, "My vision is this will be the quantitative tool of choice to allow you to more easily measure" actin and other proteins' dynamics in vivo.

Tracking turn-ons

Following the behaviors of many proteins involved in motility also requires tracking their activation status. Knowing where in the cell and at what time signaling proteins become activated is key to understanding how cells coordinate movement.

A Scripps team led by Klaus Hahn recently did this using a technique called fluorescence resonance energy transfer, or FRET (*Science,* 13 October 2000, p. 333). The researchers created a biosensor to track active Rac, a signaling protein that initiates the formation of lamellipodia, by attaching a fluorescent label to a fragment of a protein that binds Rac when it is activated. In addition, they linked Rac to another fluorescent tag, called green fluorescent protein. GFP fluoresces at a wavelength that excites the biosensor label. So when the two proteins are in close proximity, as occurs when Rac is active, researchers can see the biosensor shine. By introducing both proteins into cells and tracking GFP fluorescence, the researchers showed that stimuli that induce migration turn on Rac at the cell's leading edge.

Although FRET is not a new technology, its use has been limited because it's difficult to get it to work. "Engineering things so that you actually get useful information and don't perturb the biological system is what's hard," says Martin Schwartz, who collaborated with Hahn and also works at Scripps. Schwartz hopes to create biosensors to track the activities of several proteins involved in migration.

Knock-in, knock-out

Light-sensitive molecules are useful not only for following proteins but also for manipulating them. "We can either selectively activate or knock out certain molecules in a very precise spatial and temporal way," says Ken Jacobson of the University of North Carolina, Chapel Hill. Jacobson's team made migrating cells turn by shining a spot of ultraviolet light on one side of their lamellipodia, the researchers report in the May 2001 issue of the *Journal of Cell Biology*. The trick was that the cells were loaded with a protein that sops up actin monomers and that the researchers had chemically modified to make light-sensitive. The protein remained inactive, or "caged," until stimulated by ultraviolet light. When the spot of light "uncaged" the protein in a small region of the cell, it created an imbalance of actin monomers, causing the cell to turn about the irradiated spot. Jacobson is now working on caging other molecules that alter actin dynamics. The approach, he hopes, will clarify the roles of potential migration proteins that have been studied extensively in vitro but whose in vivo roles remain uncertain.

A complementary technique called chromophore-assisted laser inactivation (CALI) may help dissect the functions of other proteins involved in migration by allowing researchers to knock out specific proteins. Although CALI is not a new technique, Jacob-

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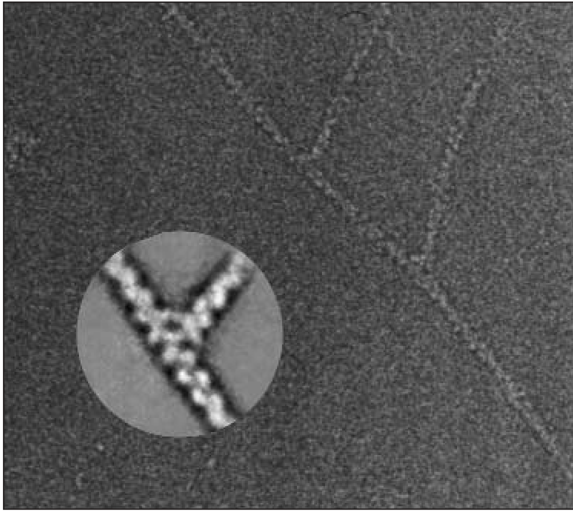


Sturdy scaffold. Researchers are trying to determine how the Arp2/3 complex allows actin filaments to branch.

son thinks it now has a particularly bright future based on his as-yet-unpublished studies showing that GFP, which is readily available and easily linked to cellular proteins, can be used as CALI's hit man. "It could be a tremendous boost in the usefulness of GFP," says Jacobson.

Pushing and pulling

Few cellular behaviors are as visibly shaped by mechanical forces as cell migration. Yet much remains unknown about how movement results from the integrated effects of cell adhesion, contraction, and the physical characteristics of the substrates cells migrate on. Taking advantage of several recent advances in technologies to apply and measure mechanical forces, however, researchers are beginning to put the pieces together.

By adapting photolithographic methods similar to those used in the semiconductor industry, Donald Ingber's group at Harvard Medical School and Children's Hospital in Boston has examined the role of mechanical stress in determining a cell's choice of direction. The team created surfaces with circular and square islands similar in size to a single cell. Their unpublished results show that when the islands are coated with extracellular matrix proteins, cells spread out to assume the shape of the island, regardless of whether the island is a square or a circle. Round cells extend lamellipodia in random directions, the team found, but square cells send out extensions primarily from their corners. "Imagine you have a little water balloon," Ingber explains. "In a circle, it's spreading out equally in all directions. But if you keep trying to pull it out on a square, the corners get stretched more than the sides. So by changing the shape, you've constrained where the cell puts its adhesions. And that constrains where it applies stress, which apparently dictates where it migrates."

Benjamin Geiger's team at the Weizmann Institute of Science in Rehovot, Israel, has also used lithography to generate patterned substrates. But they used the substrates to measure forces rather than control them. Previous experiments had shown a give-and-take between adhesions and traction forces. Mature adhesions generate traction by gripping the extracellular matrix and triggering contraction. But at the same time, mechanical forces, including contraction, shape the strength and assembly of adhesions.

In a study published in the May 2001 issue of *Nature Cell Biology*, Geiger's group placed cells sporting fluorescently labeled adhesion proteins on flexible substrates featuring arrays of tiny pits or bumps whose exact positions could be tracked under the microscope. They were then able to monitor in real time how the cells gripping the substrate deform the arrays and consequently obtain precise measurements of the local forces the cells exerted at the adhesion sites. The researchers found that the larger the force, the larger the associated adhesion complex. And when they applied a drug that blocks contraction, the adhesions shrunk within seconds, suggesting that contraction affects adhesion assembly almost instantaneously, perhaps by directly deforming or rearranging adhesion molecules and their associated actin filaments.

Virtual movement

Until recently, most cell biologists had shied away from theoretical approaches, often arguing that the complicated nature of cell behavior was not amenable to mathematical descriptions. But a growing number of studies are showing how computer modeling can help answer questions ranging from how actin pushes the cell membrane forward to how cells use chemical gradients to steer their crawling. More biologists are welcoming the technique as a valuable asset. "Our intuition about how things work is frequently faulty and can often only be appreciated after making a mathematical model," says Pollard, a biologist who has long collaborated with modelers.

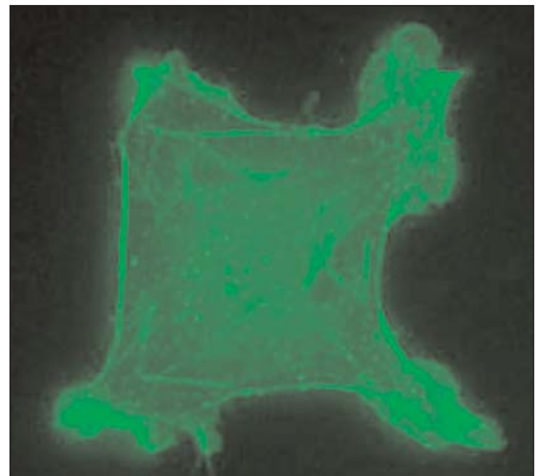
Despite modeling's growing popularity, several hurdles block its widespread use. Most agree that to be truly informative, theory and experiments must go hand in hand. Yet biologists often lack the mathematical training to create their own models, and setting up multidisciplinary collaborations is not always easy. One approach that may give biologists a hand is being developed by Alex Mogilner of the University of California, Davis, and Leslie Loew of the University of Connecticut Health Center in Farmington.

"What we hope to do is to make a computational tool that will allow ordinary biologists to simulate moving cells on the computer," says Mogilner. "When we model, we do a lot of esoteric stuff that people who are not trained in mathematics cannot do. But if we're successful, then basically any scientist with only an elementary training in mathematics will be able to do it."

Based on software developed by Loew, called Virtual Cell, the researchers hope to create a program in which users can test hypotheses about how different parameters—including cell shape, the number and distribution of cellular components, ion and protein concentrations, and reaction rates—influence cell motility. For example, a researcher who suspects that the concentration of actin monomers at the leading edge helps determine cell speed could use the model to obtain an estimate of the range of concentrations that are likely to affect speed in a real cell and then design experiments accordingly. "Of course, a lot of [the parameters are] unknown," explains Mogilner. "But that's the role of the model: You play with the concentrations and rates and see what the model gives you as an outcome."

The study of cell migration seems poised for integration, with researchers using a variety of approaches beginning to find common ground. "It's kind of like the railroad in the 1800s: people working from two coasts and meeting around Salt Lake City," says Jacobson. "We hope the top-down approaches meet the molecular approaches and give us some understanding of how these processes are integrated." –MARINA CHICUREL

Marina Chicurel is a writer in Santa Cruz, California.



Stretching out. A square substrate puts stress on a cell's corners, determining where it builds lamellipodia.

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