Exploring the building blocks of cell movement, researchers are revealing delightful dances—and changing dogma.

by Elise Lamar • illustration by Jamie Cullen
The cytoskeletal network of a cell is somewhat similar to an animal skeleton: it provides a scaffolding and a means for stepping forward. But unlike a bony skeleton, the cytoskeleton works only when it is unstable. Most locomoting cells move not by discrete steps but through continuous scaffold extension on the front end and destruction at the rear—a process sometimes likened to a treadmill.

The primary constituent of the scaffold is the protein actin—a molecule that never sits still. As the cytoskeleton extends, cells spin single actin molecules into long chains, or polymers, aided by a stew of molecular bundlers, cross-linkers, and branchers. The actin scaffold supports whatever protrusion a cell needs to crawl or pry its way through tissue. Disrupt the balance of construction and demolition and the cellular healers are going nowhere. Neither are metastasizing cells.

Given its importance, the cytoskeleton seems an obvious target for drug discovery. But the very ubiquity of cytoskeletal proteins has raised doubts about whether actin or any of its handlers could serve as pharmaceutical targets. “One prejudice has been that because cytoskeletal proteins are inside cells and abundant, they are undruggable,” says Joan Massagué, cancer researcher and HHMI investigator at Memorial Sloan-Kettering Cancer Center.

But recent findings by HHMI scientists and others reveal that the cytoskeletal architecture differs significantly from cell to cell. “Immune cells and neurons put their Legos together in completely different patterns,” says HHMI investigator Julie Theriot, who studies cell motility at Stanford University. If cells display specialized cytoskeletal structures, researchers have options for speeding the rescuers or blocking the invaders in a targeted way. In other words, the “undruggable” dogma is crumbling.

MOVING FORWARD

Topping the “Greatest Hits” page of Theriot’s lab website is a video of disease-causing *Listeria monocytogenes* whirling around inside canine kidney cells. The cells are engineered to express fluorescent actin, and the bacteria inside them appear to stream a glowing “comet tail.” But the tail actually represents dissolving actin filaments constructed by the host cell, whose cytoskeleton has been coopted by the bacteria to propel themselves through infected tissue.

“The comet tail video shows that the cytoskeleton is a powerful machine constantly running, poised to push things around,” says Theriot. “All a bacterium needed was to figure how to tap into it.” Her group discovered that a single surface protein expressed by *Listeria* was sufficient for “tapping into” the dynamic actin cytoskeleton and could generate comet tails when inserted in unrelated bacteria, or even plastic beads. The bacterial protein works by latching onto a host cell actin-binding protein complex called Arp 2/3, an actin “brancher.” Once that happens, the Arp 2/3 complex stimulates growth of a new actin filament from the side of an existing filament, generating a branched structure that first pushes *Listeria* forward and then disintegrates in its wake.

Rather than conspire with bacteria, the primary purpose of the actin engine in a human cell is to move that cell to a specific location where it is needed. HHMI early career scientist James Bear at the University of North Carolina at Chapel Hill is trying to figure out what controls the migration by studying connective tissue cells called fibroblasts.

After an injury, chemical cues emanating from a wound lure reparative fibroblasts in a process called chemotaxis. Cells migrate toward the wound guided by a flat, foot-like structure known as the lamellipodium, from “lamella” (thin sheet) plus “podium” (foot). Lamellipodia constantly probe forward, advancing a cell by means of the persistent cytoskeletal engine as it assembles and dismantles actin branches. Until recently, many investigators believed that lamellipodia might also interpret chemical signals released from wounded tissue. But a study from the Bear lab published March 2, 2012, in *Cell* shows it’s not that simple.

Bear genetically engineered fibroblasts without lamellipodia by depleting cells of the Arp 2/3 complex, blocking their ability to make highly branched actin. He then exposed the cells to traces of multicyclic organisms harbor armies of cells on the move. Most are on goodwill missions—immune cells chase bacteria, and wound-healing fibroblasts rush in to fill gaps after injuries. Others, such as metastatic cancer cells, travel with deadly intent. The biochemical signals that set cells on a journey are as diverse as the tissues they move through, but the engine is driven by constant remodeling of a protein network built from a box of cellular Legos.
of a growth factor “lure” normally secreted from wounds. Even though their primary means of locomotion had been cut out from under them, the cells were able to move toward the growth factor using less efficient protrusions.

“This was a surprise—everybody in the field assumed Arp 2/3 was essential for chemotaxis,” says Bear. On the other hand, the researchers reported, the loss of Arp 2/3 did adversely affect the ability of the cells to sense and respond to the surface they crawled over.

The take-home message is that coordinating external signals with cytoskeletal rearrangement is astonishingly complex, which could be good news: the more complex the process, the more opportunities for intervention. Take cancer cells, for example. Bear points out that in terms of motility, metastasizing cancer cells, though frighteningly effective, may just be generalists. “Metastatic cells are like the winners of the decathlon,” he says. “They have to win 10 different events but only passably well.” Tripping over a hurdle may be sufficient to put them out of the game.

THE SPECIALISTS

Actin and its brancher, the Arp 2/3 complex, are the nuts and bolts of the cytoskeleton and therefore may not be good starting points for designing drugs to perturb motility in a targeted way. Better candidates may be found in specialized actin bundlers or cross-linkers, which mold the scaffolding underlying specialized “feet” and other protrusions.

Among the bundlers is a group of proteins called coronins. In successive Cell articles, published in 2007 and 2008, Bear reported that an actin-binding protein called coronin 1B controlled the extent of actin branching by putting the brakes on the Arp 2/3 complex. Without coronin 1B, the cytoskeletal network was elaborate but rigid, causing fibroblasts to move more slowly—a big liability for a wound healer.

A different coronin, subtype 1A, appears critical for avoiding catastrophic immobilization of immune cells. Collaborating with Bear, HHMI investigator Jason Cyster reported in a 2008 Nature Immunology paper that in mice with a mutation in the gene encoding coronin 1A, T lymphocytes could not exit their birthplace, the thymus, to activate an immune response in peripheral tissues.

“These mutant mice have no peripheral T cells and were highly immune compromised,” says Cyster, of the University of California, San Francisco (UCSF). The researchers also reported that a patient with severe combined immunodeficiency, or SCID, had coronin 1A mutations, suggesting that perturbing actin branching in a way that paralyzes cells is not insignificant but rather promotes a deadly disease.

Metastasizing cancer cells show protrusions reminiscent of lamellipodia but they are likely specialized for specific cellular environments. Some cancer cells express high levels of yet another coronin, coronin 1C, suggesting that changes in actin branching may enhance tumor cell invasion. Bear’s lab is examining cultured cells and animal models to determine whether upregulation of coronin 1C stimulates actin cycling in a way that enhances motility in human melanoma cells.

For most of his career, cancer researcher Massagüe has investigated signals that fire up the cytoskeletal engine; he is also
evaluating the effect of actin-interacting proteins on metastasis. In 2005, his lab group identified 18 “signature” genes associated with lung metastasis of human breast cancer. Most of them encoded factors that cells use for communication, like cytokines and their receptors. But one, called *fascin*, encoded a protein that bundles actin filaments into rods supporting spiky protrusions called invadopodia (picture lean lamellipodia armed with pickaxes). Blood and muscle cells occasionally use invadopodia to grasp a surface, but they are most common in tumor cells.

“Cells use fascin protrusions to pry through layers of cells—for example, those lining lung capillaries,” says Massagué. “It makes complete sense that breast cancer cells would find a way through the bloodstream into the lungs by augmenting invadopodia power.”

Japanese scientists seeking tumor inhibitors based on natural products have identified an anti-fascin molecule called migrastatin from *Streptomyces platensis* bacteria. Massagué and chemist Samuel Danishefsky, of Columbia University and Memorial Sloan-Kettering Cancer Center, have teamed up to create and test potent migrastatin analogs to slow movement of metastatic cells; in work published September 13, 2011, in the *Proceedings of the National Academy of Sciences*, Danishefsky reported that some of those analogs effectively block metastasis to several sites, including liver, heart, kidneys, and spleen, in a mouse model of human lung cancer.

A direct link between coronins and metastasis has not been confirmed, nor have migrastatin-type drugs been tested in a clinical setting. But two exciting concepts emerge from these studies: One is that actin accessory proteins modulate cytoskeletal rearrangements related to the motility of either healers or invaders. More significantly, these factors are diverse in the way they bind to and mold actin filaments, suggesting it may be possible to tinker with one interaction without perturbing another.

### THE DIRECTORS

Targeting actin accessory proteins such as coronins might be a viable strategy in some immune disorders. In their coronin 1A mouse mutants, Cyster’s team showed that the signaling apparatus that lymphocytes use to find their way out of the thymus, a receptor called S1PR1, was intact. Yet cells remained paralyzed, as they couldn’t move their lamellipodia because of coronin defects, a situation analogous to the crippled chemotaxis displayed by Bear’s lamellipodia-less fibroblasts. These experiments suggest that the converse may also be true—the motility of cells with a perfectly normal cytoskeleton could be halted if the signals regulating it are blocked.

The signal detected by the S1PR1 receptor is a lipid called sphingosine-1-phosphate (SIP), present in blood and lymph. Cyster’s group has shown that when mature immune cells are ready to leave lymph nodes to travel to target sites, they move toward node exit doors by detecting faint traces of SIP in the circulation via the S1PR1 receptor. In work published September 30, 2011, in *Science*, his lab demonstrated the converse: that the receptor temporarily shuts down when immature cells need to get back in to the node.

In 2010, the FDA approved use of a fungal derivative drug called fingolimod (FTY720) to treat multiple sclerosis, a condition characterized by an autoimmune response against cells of the patient’s own nervous system. Chemically, fingolimod resembles SIP and likely works by acting as a decoy and binding to S1PR1, jamming its deployment signals to the cytoskeleton. “The current hypothesis is that FTY720 acts as an immunosuppressant by inhibiting lymphocyte egress from lymph nodes,” Cyster says. But he cautions that other mechanisms are also possible.

### MOVING BACKWARD

Yale University cell biologist Tom Pollard is a pioneer in cytoskeletal research. Not only did his lab group discover the Arp 2/3 complex, his team was also the first to image fluorescently labeled actin filaments forming in real time. He remembers his fascination with amoebas in high school in the 1950s. “Back then I wanted to be a gremlin inside cells to see how these things happen.”

Four decades later, just such a gremlin would testify that crawling cells first advance some kind of protrusion, lean forward to extend it (often by actin branching), simultaneously demolish the rear scaffolding, and then let go and scrunch forward.

Lavish attention has been paid to step one, in part because protrusions exhibited by motile cells from amoebas to white blood cells called neutrophils are often big, easy to image, and highly photogenic. But Theriot is addressing the equally critical but much less documented “anti-event”—namely, how the back end of the cell lets go. To do so she studies keratocytes, highly motile cells that are found in the basal layer of the epidermis. As a model, Theriot uses fish scale keratocytes, which rapidly repair skin lesions.

Like fibroblasts, keratocytes project a lamellipodium filled with branched actin. But in 2010, Theriot reported in *Nature* that deconstruction of that meshwork, a process necessary to keep the...
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Larry Zipursky

treadmill moving, required recruitment of a form of myosin—a motor protein filament common in muscle—to the actin cytoskeleton at the rear of the cell, which literally ripped the actin fragments apart. Without that destruction, cells couldn’t move because their cytoskeleton was too stable, analogous to how coronin loss slows cells by making the cytoskeleton overly stiff.

How rapidly the cytoskeleton undergoes cycles of construction and demolition directly determines cell speed, which in keratocytes is roughly a fraction of a micron per second. Factoring into that equation is tissue adhesiveness. “If adhesion is too low, myosin activity keeps a cell running in place,” explains Theriot. “But on a surface that is too sticky, keratocytes have difficulty pulling up their backside to glide along.”

In a 2011 follow-up PLoS Biology study, Theriot quantified every move a keratocyte makes on “sticky” versus smoother surfaces—how fast actin filaments form and dissolve, how much traction the cell gets, how its shape changes—so she could calculate cell speed in various microenvironments. “Cells of the immune system may travel through the bloodstream, inflammatory environments, or layers of epithelial cells where things could get stickier,” she says. Knowing how to calculate speed through different tissues could come handy when devising ways to speed up cells or stop them in their tracks.

MARCH OF THE GROWTH CONES

Orkun Akin started his career playing soccer with the actin cytoskeleton. As a UCSF graduate student working with R. Dyche Mullins, Akin used a cell-free system to analyze motility by tweaking concentrations of actin, Arp 2/3 complex, and other actin-binding proteins. Without the boundaries of a cell, he measured “motility” based on how well the “motility mixture” kicked around a polystyrene bead in a dish, similar to propulsion of Listeria. His observations, published in Cell in 2008, suggest another mechanism of actin branching.

Now a postdoctoral fellow in the lab of HHMI investigator Larry Zipursky at the University of California, Los Angeles (UCLA), Akin is imaging the cytoskeletal machines guiding nerve cell axons, to form synapses—neutral connections—between R8 photoreceptors in the Drosophila eye and the fly’s brain. During synapse formation those machines, called growth cones, creep forward, seeking the right target.

Zipursky’s group and others are making headway in understanding the cell surface receptors, signaling molecules, and cytoskeletal proteins that regulate growth cone movement in the fruit fly. Much of the work on the developing visual system has drawn on the power of the fly model, which allows scientists to genetically manipulate specific neuronal cell types. A major limitation to linking gene function to growth cone motility, however, has been the lack of a robust system for visualizing growth cone movement in live animals.

To address this challenge, Akin teamed with UCLA neurobiologist Joshua Trachtenberg to build two-photon microscopy to follow development of R8 growth cones in flies. The work led to the creation of a remarkable video of R8 growth cones forming connections in the intact animal. First, some 750 amorphous R8 photoreceptor growth cones glowing green with actin filaments hover like a fleet of Close Encounters spacecraft over the optic lobe landing pad. Some hours later, each R8 growth cone extends a spiky, fluorescent finger-like extension, a filopodium, into the region of the optic lobe where it will make synapses. This action is followed by extension of the rest of the axon to the same region.

“It was a big surprise that we could look through a fly pupa and see R8 cells developing in synchrony,” says Zipursky. “When I saw that, I knew we had something unique.”

Akin will now determine how that choreography is disrupted in flies with mutations in various signaling proteins. “Growth cones go from a stalled morphology abruptly to a moving state and then stop again,” says Akin. “We know the signal that activates the movement and its receptor, and now we want to know what role actin dynamics plays in this transition and how signaling factors regulate that process.”

Clinical applications of the photoreceptor work are far off, but Zipursky sees obvious relevance to stem cell–based replacement therapies aimed at regeneration. “Knowing how to wire neurons up properly will require knowing what’s going on biochemically inside a growth cone,” he says.

And the success of the multiple sclerosis drug fingolimod, which alters cell motility, suggests that this goal is not unrealistic. The factors that drive the cytoskeletal engine are not, in fact, undruggable. Engine components themselves, like coronins and fascins, could be next on the list.

Akin, buoyed by the youthful optimism that drove him to build a microscope, agrees. “The more we focus on specific cell types, the more insight we may gain about whether cells in any disease model have a cytoskeletal Achilles heel.”

WEB EXTRA: To see the cells described in this story in motion, visit www.hhmi.org/bulletin/may2012.

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