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Microtubules as a gyroscope for cells on the go

Free advice never hurt anyone. And when Harry Malech couldn’t decide between research on cell motility or infectious disease, his fellowship mentor Richard Root offered up this gem: “You know how to combine that, don’t you? You ought to be interested in neutrophils—they are the fastest cells alive.”

So began a study of neutrophil chemotaxis that became the first clear demonstration that microtubules oriented and organized the internal structure of migrating cells (Malech et al., 1977). In an earlier study, Goldman (1971) had noted that after destruction of microtubules with colchicine “no one ruffling edge [of a motile cell] seems to be capable of taking over as the leading edge,” suggesting that these “fibers may be involved in determining which ruffling edge becomes the leading edge.” But a dynamic picture of such a process was missing. The work also showed that migration and pseudopod formation required functioning actin filaments. While many experts had postulated various roles for the two major classes of cytoskeletal filaments, the study clarified their distinct, yet complimentary, functions in migration.

“The study” of white cell locomotion at that time was very descriptive, says John Gallin, who teamed up with Malech and Root. “In response to a chemoattractant, was there a rudder? A skeletal structure that gave it shape? I was thrilled that we could use cells from humans to demonstrate this.”

Malech had been setting up “chemotaxis chambers” with porous filters that neutrophils could migrate through in response to a toxin-activated serum gradient. He would then embed the whole filter with cells in resin for sectioning and electron microscopy. On one “serendipitous” day, Malech says he grabbed the wrong size filters, with pores too tiny for whole cell migration. He didn’t realize his mistake until he looked at the sections and “was amazed to see these cells lined up like little soldiers in frustrated chemotaxis.”

He realized the accidental assay would make it easy to ask what was common about the immobilized cells trying to move in the same direction. When he reversed the direction of the chemoattractant, he observed that the positions of the nucleus and centriole and orientation of microtubules also reversed rapidly.

When he added cytochalasin B to disrupt actin filaments, the neutrophils could no longer migrate toward a reversed chemical gradient and failed to put out pseudopods. Yet their internal organelle structure still shifted in response to the gradient reversal. Adding colchicine, the microtubule inhibitor, had roughly the opposite effect—random migration was not impaired but the organization of internal structures broke down. And, without microtubules, attractant-directed migration became severely handicapped. “like a drunken man who can’t walk a straight line,” says Malech, now chief of the Laboratory of Host Defenses at the National Institute for Allergy and Infectious Disease (Bethesda, MD).

The study hit home the point that microtubules’ role in migration is similar to how “a gyroscope keeps a moving vehicle on course,” says Malech. “It’s not the motile force or the steering wheel, but it provides stability by giving the direction of which way [the vehicle] is going and has been going.” Malech and Gallin extended their study of neutrophil microtubules into clinical applications like the study of Chediak-Higashi syndrome, a rare disorder involving recurrent infections downstream of neutrophil dysfunction (Gallin et al., 1980).

Gallin, J.I., et al. 1980. Ann. Intern. Med. 92:520–538.
Goldman, R.D. 1971. J. Cell Biol. 51:752–762.
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Actin and microtubules interact via MAPs

Circumstantial evidence rarely stands up in a court of law. In science, correlation has a little more credence, especially when it leads to a testable hypothesis. By 1978, people had been studying microtubules for a dozen years and nonmuscle actin filaments for about nine years. There were “plenty of examples in electron micrographs where microtubule and actin filaments were in the same place in the cell,” says Thomas Pollard (Yale University, New Haven, CT).

But, he notes, no one had investigated whether the polymer filaments actually interacted with each other and, if so, by what molecular connections. After seeing more provocative but still circumstantial images at a summer Woods Hole Marine Biological Laboratory course, Linda Griffith, then a graduate student at UCLA, asked Pollard if she could transfer to his Harvard laboratory to test the idea.

The two used a rather low-tech viscometer that measured a ball bearing’s rate of fall through a capillary tube filled with actin filaments and microtubules (Griffith and Pollard, 1978). Pollard says the idea came from engineers at MIT who were using the falling ball method for other purposes. This simple method gave the team an easy biochemical assay, which caused less shearing of the filaments than traditional capillary viscometers.

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Basal lamina instructs innervation

Look Ma, no cells. In what he calls “a hyper-low-tech experiment” by today’s standards, Joshua Sanes was in 1978 able to show that regenerating nerve axons take their cues for new synapse formation from the extracellular matrix (ECM) of muscle cells and not from the cells themselves (Sanes et al., 1978). The beautiful simplicity of the experiment gave scientists studying synapse formation a clear trail to follow to find the signals for axon guidance and differentiation.

From the late 19th century studies by a student of Santiago Ramón y Cajal, it was known that a cut nerve would form a new synapse at the same exact spot on the muscle fiber where the old synapse had been.

“Somehow, the axon is ignoring 99.9% of the muscle fiber and contacting the 0.1%,” says Sanes. “It had to be recognizing something on that spot—a surface component or a chemical.” He and his colleagues reasoned that the regrowing axon first contacts the muscle cell’s extracellular matrix sheath, which was called the basement membrane because it was thought to be the support foundation for muscle cells.

The group devised a way to observe damaged frog muscles where axons, but not muscle cells, would grow back. The team cut muscle and nerves in a patch of tissue and then irradiated it so that damaged muscle would not grow back, but damaged nerves with cell bodies outside of the irradiated patch could regenerate into the damaged area. All that remained of the damaged muscle cells were the basement membrane sheaths, made of insoluble glycoproteins that persisted up to four weeks later.

These “ghost” sheaths could be stained for a synapse marker, cholinesterase, showing where previous synapses of the neuromuscular junction had existed. The team’s EM pictures unmistakably show new nerve terminals forming next to the darkly stained cholinesterase.

Sanes says that although “no one ever thought Nobel, we did feel it was cool and would be helpful in this field.” Indeed, the work showed the ECM to be more than an inert structural support. It was instead playing a clear developmental role—all this at a time when ECM components were just coming to light. Practically, the study also gave Sanes, his coauthor Jack McMahan, and others a narrowly defined location for their search for molecules that would be helpful in this field. Indeed, the study also gave Sanes, his coauthor Jack McMahan, and others a narrowly defined location for their search for molecules that would be helpful in this field. McMahan’s group pursued and identified the ECM molecule agrin that was deposited by motoneurons and “spoke” to the postsynaptic muscle cells (McMahan, 1990). And Sanes’s lab discovered that ECM-localized laminin β2 directed differentiation of synapses during both regrowth and development (Hunter et al., 1989).

The team has also applied lessons from that system in understanding brain synapses. Their latest contribution has a familiar theme but with a twist: only the correct ECM components are critical during normal synaptic development in vivo (Noakes et al., 1995; Gautam et al., 1996). “This has been one main thread in my lab from work started in that paper,” he says. “We’ve continued to work in synapse formation at the neuromuscular junction synapse” for the last 25 years. The team has also applied lessons from that system in understanding brain synapses. Their latest contribution has a familiar theme but with a twist: only the correct ECM components, not the nerve cell, is required for the muscle cell to set up its own synapse architecture (Kummer et al., 2004).

Gautam, M., et al. 1996. Cell. 85:525–535.
Hunter, D.D., et al. 1989. Nature. 338:229–234.
Kummer, T.T., et al. 2004. J. Cell Biol. 164:1077–1087.
McMahan, U.J. 1990. Cold Spring Harb. Symp. Quant. Biol. 55:407–418.
Noakes, P.G., et al. 1995. Nature. 374:258–262.
Sanes, J.R., et al. 1978. J. Cell Biol. 78:176–198.

The ECM sheath left behind after muscle degeneration (top, lining cavity) can direct development of a new synapse (bottom, dark stain).

Microtubules and actin filaments line up next to each other in vitro.

When they added purified tubulin and actin to the tube and allowed filaments to polymerize, the viscosity was similar to the sum of the filaments’ individual viscosities. But when microtubules were purified with their microtubule-associated proteins (MAPs), the viscosity of the mixture with actin jumped 120-fold. The experiments argued heavily for MAP-mediated cross-linking of microtubules and actin filaments.

The paper was one of the first to assign a molecular role to the MAPs beyond promoting microtubule polymerization. A few years later, Pollard’s team showed that phosphorylation of MAPs inhibited the actin filament interaction (Selden and Pollard, 1983). Recently, microtubule–actin interactions have made headlines again, with advances in light microscopy revealing that the filaments interact at the leading edge of migrating live cells (Rodriguez et al., 2003).

Griffith, L.M., and T.D. Pollard. 1978. J. Cell Biol. 78:958–965.
Rodriguez, O.C., et al. 2003. Nat. Cell Biol. 5:599–609.
Selden, S.C., and T.D. Pollard. 1983. J. Biol. Chem. 258:7064–7071.