People & Ideas

Dyche Mullins: Finding filaments at the fringes

Mullins has made his mark by exploring the actin cytoskeleton's frontiers.

Dyche Mullins morphed from an electrical engineer to a cell biologist during his doctoral work at the University of Kentucky. Attending the intense Marine Biological Laboratory (MBL) Physiology Course assured his transformation and earned him a postdoctoral spot with the course director at the time, Tom Pollard. During his time in Pollard's lab, first at Johns Hopkins University and then at the Salk Institute for Biological Studies, Mullins discovered the role that the Arp2/3 complex plays in nucleating branched actin networks. After establishing his own laboratory at the University of California, San Francisco, at the end of 1998, Mullins's team discovered another actin nucleation factor, Spire. Mullins returned to Woods Hole, Massachusetts, to lead the Physiology Course in the summers of 2009–2013, codirecting with his former lab partner Clare Waterman.

Lately, his group has been preoccupied with actin's unorthodox side: the mysterious functions of nuclear actin (1), the connection between actin assembly and DNA damage–induced cell death (2, 3), and the mechanisms that actin-like proteins (ALPs) found in bacteria use to push plasmids around (4). Mullins spoke with *JCB* about his recent collaboration with Nobel-winning microscopist Eric Betzig (5) and how his name might have destined him to investigate the structural engineering inside cells.

> "I like moving into the crazy border town."

ORIGIN STORIES

What is the origin of your name?

So many people ask about it that I looked it up in the *Oxford English Dictionary*. It's the Middle English spelling of a word that meant "earthworks." It bifurcated at some point into the words "ditch" and "dike." It's also a family name in eastern Kentucky where I grew up. I've always assumed that I was named after a very long and illustrious line of ditchdiggers.

What turned you toward cell biology?

My first summer in graduate school for electrical engineering, I worked for a biologist, Betty Sisken, building equipment for her lab. I remember the moment I discovered that there was such a thing as the calcium ATPase—that a single molecule could be a very sophisticated machine that can burn energy and pump calcium across a gradient. That just seemed like the most fantastical thing I could imagine. That's when I realized that the problems I was going to find interesting were in biology.

Recently you've been interested in the function of nuclear actin…

There was no technology to specifically perturb actin in the nucleus without affecting the cytoplasm. And there were no tools to look at actin specifically in the nucleus. So we developed some. Graduate student Brittany Belin did an amazing job of designing tools for looking at nuclear actin, both monomeric and filamentous, in live cells. She's ruthlessly systematic. She took every actin-binding domain that she could find in the literature and she fused that to both GFP and a strong nuclear localization signal and then screened them all.

One of her probes illuminated short nuclear actin filaments that appeared to backtrack. What's going on there?

We were trying to determine whether those filaments exhibited directed motion or random motion. If there were motors causing things to move along a linear track, you would see a directional signal at short timescales. But what we got was the opposite. At short timescales, the filament was more likely to be going in the opposite direction of its previously recorded direction.

Others have found a similar behavior for particles diffusing in the extremely crowded bacterial cytoplasm. They called it fractional Langevin motion. The nucleoplasm is so densely crowded that, to move to any new place, you have to displace other molecules. If those displaced molecules are connected in some large-scale way, they act like an elastic medium, or a spring that pushes back against a diffusing molecule.

PHOTO COURTESY OF DYCHE MULLINS

Dyche Mullins

DUAL-PURPOSE ACTION

What did you think when you found that the nuclear p53 coactivator, JMY, was also nucleating actin in the cytoplasm?

In one sense I was relieved to think that maybe we wouldn't have to worry about its nuclear functions—we'd let the transcription people worry about that.

We showed that JMY has an intrinsic actin nucleation activity almost indistinguishable from that of Spire. Former grad student Brad Zuchero saw JMY near leading edges and ruffling membranes, especially in fast-moving neutrophil-like cells that had been polarized by a chemoattractant. It was mainly in undifferentiated and/or nonmotile cells that we found JMY in the nucleus. We and others also found that JMY enters the nucleus in response to DNA damage.

When did you realize that JMY's nuclear role was connected to its role as an actin regulator?

In the process of crossing t's and dotting i's to show that JMY enters the nucleus in response to DNA damage, as had been previously shown, Brad discovered that it was actually the cytoplasmic actin monomer concentration that drives it into the nucleus. Then we found that the WH2 domains that bind to actin overlapped with JMY's nuclear localization sequence.

It turns out that, if you induce DNA damage in cells, they polymerize a lot of actin in the cytoplasm—the amount of polymer

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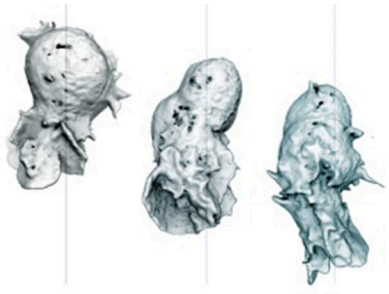

IMAGE COURTESY OF LILIAN FRITZ-LAYLIN, MEGHAN RIEL-MEHCAK, TOM FERRIN, AND TOM GODDARD

Surface rendering of a neutrophil-like HL60 cell observed by lattice light-sheet time-lapse imaging.

almost doubles, which means the monomer concentration drops precipitously. Almost nothing is known about this DNA damage–induced actin assembly pathway. But it seems like some primary response to stress that triggers JMY to go to the nucleus, where it initiates p53-mediated apoptosis.

Why would actin polymerization be linked to DNA damage in this way? To move cells away from potential dangers?
Actually, there is less ruffling and cells become less motile. They are incorporating a lot more actin into stable structures like stress fibers. Maybe it is more like the cell hunkering down. But again, this has not been well studied.

JMY is a bit like that old Saturday Night Live skit: "It's a floor wax *and* a dessert topping."

PRIMORDIAL CREEP

What drew you to bacterial actin-like proteins?
I really like fields that are wide open, or fields that are in chaos and need some work to tame them. I like moving into the crazy border town without a sheriff.

When Jeff Errington's group definitively showed in 2001 that there were filamentous actin-like polymers in bacteria, it was hugely appealing to me. Here was a wide open vista. We could read one paper and know as much as anyone else in the world about this topic. This was the time to get into a field like this.

What do these ALPs do in bacteria?
MreB is involved in cell wall synthesis, but, after the discovery that it forms filaments, several groups began looking for, and finding, additional actin-like proteins in bacteria. Joe Pogliano set the current world record by identifying almost 40 different classes of such proteins. We have demonstrated that several of them do in fact form filaments, but in most cases we have no idea what they do.

Some actin-like proteins are involved in segregating low-copy bacterial plasmids. For example, if there are only two copies of a plasmid per bacterial cell, the chances that both will end up on the same side of the division plane are pretty high—especially given their *very* low mobility. Plasmids in cytoplasm are like peas stuck in Jell-O. Assembly of actin-like proteins—which are encoded on the plasmid—can be harnessed to push two plasmids apart so that, when the cell divides, each daughter cell gets at least one copy.

We worked out mechanisms by which one actin-like protein, ParM, segregates DNA, and, to our great surprise, this protein turns out to be dynamically unstable. Similar to eukaryotic tubulin, ParM filaments switch between growing stably and rapidly falling apart. This switching behavior is important because, if we mess with it, we completely screw up plasmid segregation. ParM and tubulin do not share a dynamically unstable common ancestor, so this represents a remarkable case of convergent evolution at the molecular level.

"It looked like an angry Chihuahua."

We like these actin-like proteins because they are a good playground for working out molecular mechanisms of filament behavior. I like the idea that, hidden somewhere among the bacterial and archaeal actin-like proteins, we will find evidence for the origins of the eukaryotic actin cytoskeleton.

Why do we care how bacteria shuttle their plasmids around?
Well, the R1 plasmid we worked on to study the ParM system originally came from a *Shigella* outbreak in a Tokyo hospital in the 1950s. It was an early example of a super drug-resistant bacteria. It's a really nasty plasmid to have around. We are interested in doing screens for small molecules that jam up ParM, either by causing depolymerization or stabilizing the polymers. That would either impair bacterial growth or force loss of the drug resistance plasmid.

You were an early user of Eric Betzig's lattice light-sheet microscope. How did you feel the first time you saw a crawling neutrophil using this 3D technique?
I connected with Eric and his group at the MBL in Woods Hole and then sent a postdoctoral fellow, Lillian Fritz-Laylin, to Janelia Farm to collect more data. When she showed me her first movies I was completely blown away. The cell looked like an animated scanning electron micrograph. You could see all of these unbelievable dynamics. We saw pseudopodia, with sheets and rosettes of membrane coming out and coalescing and diving around like shark fins at the front of the cell.

It looked like an angry Chihuahua. The cell body was like this big head with twitching lamellipodial ears, dragging around a little tiny uropod body behind it. I'd never seen cell movement that way before. Nobody had ever seen it that way before. The cells were not placid fried eggs but more like "cavorting wee beasties." I felt like Anton van Leeuwenhoek.

Cell biology doesn't exist without the microscope. You can learn a lot by looking at dead things, but if you want to learn about life and living systems, it helps to look at living things.

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3. Zuchero, J.B., B. Belin, and R.D. Mullins. 2012. *Mol. Biol. Cell*. 23:853-863.
4. Garner, E.C., C.S. Campbell, and, R.D. Mullins. 2004. *Science*. 306:1021–1025.
5. Chen, B.-C., et al. 2014. *Science*. 346:1257998.

IMAGE COURTESY OF DYCHE MULLINS

Mullins studies actin from multiple angles.