Matthew Welch: The many branches of actin regulation

Welch investigates how host cells and pathogens initiate actin polymerization.

The actin cytoskeleton powers a variety of processes, from cell migration to intracellular membrane transport. Pathogens often harness their host's actin network to their own ends, hijacking the cytoskeleton to drive cell entry, survival, and intercellular spread. Matthew Welch studies the many different mechanisms that host cells and pathogens use to initiate actin filament assembly at the right time and place.

Welch first became interested in actin as a graduate student with David Drubin at the University of California, Berkeley, where he used genetics to look for actin-interacting proteins in yeast (1). During his postdoc with Tim Mitchison at the University of California, San Francisco, Welch identified the Arp2/3 complex as the host factor that nucleates branched actin assembly on the surface of invasive *Listeria* to power bacterial movement through the cytoplasm (2) and found that the same complex is present in lamellipodia (3).

Since his return to Berkeley to start his own laboratory, Welch has continued to work on the Arp2/3 complex (4) and the various nucleation-promoting factors that stimulate Arp2/3 activity at different locations within the cell (5, 6). He's also continued to study how pathogens subvert actin dynamics, recently demonstrating that *Rickettsia* species express a formin-like protein to nucleate unbranched actin filaments which drive bacterial locomotion (7) and that certain viruses also can move with the help of their host's actin cytoskeleton (8). In a recent interview, we asked Welch what first nucleated his interest in actin and where his research interests are propelling him next.

> "Arp2/3 activity has to be harnessed in different places and times in the cell."

NUCLEATING INTEREST

Where did you grow up?

I grew up in a town called Mt. Kisco in Westchester County, New York. My parents still live there. My father was a statistician who worked for IBM at the Thomas J. Watson Research Center. I used to go into work with him and although he didn't work in a laboratory himself—he was more involved in the computing side of things—there were several laboratories in the building. So I kind of grew up around science. In high school I was interested primarily in chemistry because I had a really good teacher. But I also liked biology and physics.

How did you first get interested in actin?

I didn't know anything about actin or the cytoskeleton until I got to Berkeley for grad school. I'd been a molecular and cell biology major at the University of Michigan, but I'd never actually taken a cell biology course. In fact, I didn't take a cell biology class at Berkeley either, even though I teach one there now! But David Drubin, who was a new faculty member at the time, gave a great seminar about yeast actin. And I decided to rotate in his laboratory.

At that time it was clear that yeast had a cytoskeleton, but it wasn't so clear what they did with it. However, yeast had the genetic tools available to really dissect what the cytoskeleton does in eukaryotes. So I did a genetic screen to look for actin-interacting proteins. A similar type of screen had already been used for tubulin, so we thought it was a good idea to try it with actin as well. But it didn't really work out in that we didn't identify any proteins that directly regulate actin dynamics. Nevertheless, I ended up identifying a nuclear protein that turned out to be part of a transcription regulatory complex. That's kind of interesting because actin is now known to function in the nucleus in transcription and chromatin remodeling processes. But at the time it was a little bit unclear what the two proteins had to do with each other.

BRANCHING OUT

Why did you choose Tim Mitchison's laboratory for your postdoc?

Tim and one of his graduate students, Julie Theriot, had both come to give seminars at Berkeley. Looking at actin dynamics in yeast was technically difficult at that time, but Tim and Julie had done some really beautiful work developing caged actin derivatives that they could photo-activate and use to monitor actin dynamics in mammalian cells. It was really exciting.

IMAGE COURTESY OF CHRIS PATANE

Matthew Welch

Julie had also started using *Listeria* as a model system and shown that actin dynamics in *Listeria* comet tails were very similar to those in lamellipodia. At the time, people had identified many actin-binding proteins, but none of them seemed to be good candidates for nucleating actin in the cell. So I was really interested in identifying the proteins that nucleated actin assembly in both the *Listeria* "tail" and the lamellipodium.

Genetic approaches had already identified a *Listeria* protein that was necessary for actin nucleation. But it wasn't sufficient, so I set out to purify the host proteins involved. It was a long haul, and at various points I wasn't sure I was going to be successful. But eventually I decided to reconstitute *Listeria* motility in human platelet extract instead of the *Xenopus* egg extract that Julie Theriot had pioneered. That turned out to be a better starting material because it was very rich in cytoskeletal proteins, and that allowed me to purify and identify the Arp2/3 complex as the factor that nucleated actin at the *Listeria* surface.

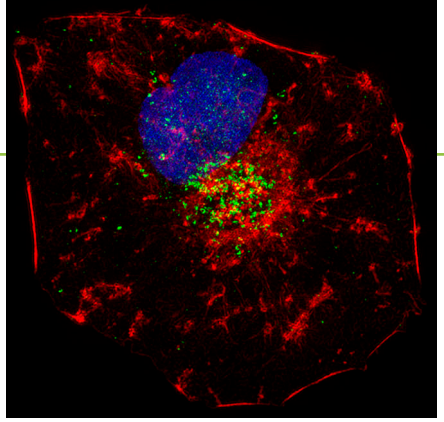

IMAGE COURTESY OF STEVE DULEH

WASH (green) stimulates the nucleation of actin filaments (red) to regulate endosomal trafficking.

Text and Interview by Ben Short
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You've investigated the Arp2/3 complex ever since. What are the most important remaining questions?

One thing that's really hindered our understanding of the mechanism of actin nucleation is that it's been very difficult to get a high resolution structure of the Arp2/3 complex in its fully activated form, where it's bound to a nucleation-promoting factor or in the branch point between two actin filaments. So I think the structural and mechanistic side of things is where we really have to make progress.

Why are there so many different nucleation-promoting factors that activate the Arp2/3 complex?

I think it's because Arp2/3 activity has to be harnessed in different places and times in the cell. Each organelle seems to require a specific factor that nucleates actin polymerization and adapts actin's function to that particular location. For example, we recently identified a nucleation-promoting factor called WHAMM, which is required for membrane transport to and from the Golgi. It's an interesting molecule because, as well as regulating actin by activating Arp2/3, it's also a microtubule-binding protein.

HIJACKING THE CYTOSKELETON

How and why do pathogens co-opt the Arp2/3 complex?

One example is during the entry process. Phagocytosis and endocytosis both involve actin, so most intracellular pathogens need to activate actin polymerization during entry. Some bacteria inject proteins into the host cell through secretion systems to activate signaling pathways or nucleate actin directly. Other pathogens express molecules that bind host surface receptors to trigger actin assembly.

Once they are in the cell, pathogens face another problem, which is how to get back out of the cell after they've replicated. That's where pathogens such as *Listeria* or *Shigella* use actin-based motility. They're good mimics. *Listeria* express the protein ActA, which directly mimics the host's nucleation-promoting factors to activate Arp2/3. *Shigella*, on the other hand, mimic the signaling pathways that activate the host factor N-WASP and use it to stimulate Arp2/3.

You recently showed that* Rickettsia *species use a different mechanism entirely…

We think they don't use Arp2/3, at least for steady-state movement. Instead, they make their own formin-like protein called Sca2 to generate parallel, rather than branched, actin arrays that drive intracellular motility. I don't know why *Rickettsia* use a different strategy, but I think it's a really intriguing question. *Rickettsia* naturally infect cells in ticks as their primary reservoir, but then they also infect mammalian cells. It's possible that the formin-type mechanism better adapts them to movement in such diverse cell types: a formin can directly polymerize actin, whereas a nucleation-promoting factor has to interface with Arp2/3, which then interacts with actin.

> "Baculoviruses… re-compartmentalize the cytoskeleton."

The other interesting thing is that there's a broad diversity of *Rickettsia* species, some of which express a very similar version of the formin-like protein Sca2 and cause diseases like Rocky Mountain spotted fever. But more distantly related *Rickettsia* species have very different Sca2 proteins that may not function like a formin. They may function like an Arp2/3 activator or an actin nucleator similar to the *Drosophila* protein Spire. So within a single group of bacteria, there's a plausible evolutionary progression of actin-based motility mechanisms.

You also investigate baculoviruses. Why are you interested in these pathogens?

Baculoviruses do really interesting things with actin. They use it for intracellular movement—which is different from other viruses, which normally use microtubules. But the really intriguing thing about baculoviruses is that they can move actin into the nucleus and polymerize it there. So they re-compartmentalize the cytoskeleton. Actin is naturally present in the nucleus, but its function there is poorly understood. Baculoviruses might offer us a window into the potential nuclear functions of actin because they may be exaggerating an existing function for actin rather than inventing a new one.

We pick pathogens to work on not because they're necessarily important causes of disease, but because they have interesting ways of interacting with actin. It's a little bit of a cliché at this point, but people say that pathogens are the best cell biologists because they've evolved over the years to exploit the workings of the cell. So they offer a window into how things work, and they're often easy to manipulate biochemically and genetically.

What are you working on at the moment?

We're continuing to focus on *Rickettsia* and baculoviruses. We're interested in exploring the evolution of actin-based pathogen motility by examining the different *Rickettsia* species. And for baculoviruses we're looking at the function of nuclear actin assembly. They may use it for replication or for completion of packaging and escape from the nucleus. Away from pathogens, we're investigating how nucleation-promoting factors direct Arp2/3 activity to different locations and processes in the cell.

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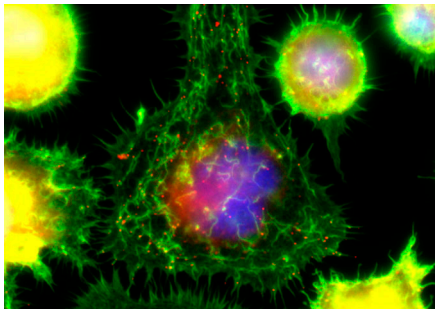

IMAGE COURTESY OF TARO OHKAWA

Baculovirus particles (red) are propelled through their host's cytoplasm by polymerizing actin (green).