People & Ideas

Jessica Feldman: Microtubule-organizing function dives off centrosomes

Feldman studies how cell patterning and cytoskeletal organization are controlled.

The microtubules of the mitotic spindle apparatus emanate from, and are organized by, the centrosomes: nonmembrane-bound organelles composed of centrioles embedded in a cloud of pericentriolar material (PCM) that persist throughout the cell cycle.

In some cell types, including fibroblasts and many cultured cell lines, centrosomes retain their microtubule-organizing center (MTOC) function during interphase. Centrioles can also be repurposed as the base upon which cilia and flagella are organized. Some differentiated cells lack cilia and flagella but nonetheless shift MTOC activity to noncentrosomal sites. Little is yet known about how this shift is achieved and regulated.

Having competed as a diver from childhood through college, Jessica Feldman has never been afraid of leaping into the unknown. From her graduate work on centrosomal positioning in *Chlamydomonas* (1, 2) to her studies on noncentrosomal MTOCs (3–5), Feldman has dived deep into the question of how cells organize themselves. She and her colleagues have made new findings regarding how the site of microtubule organization is designated in *C. elegans* gut epithelia (4, 5). We called her at her Stanford lab to learn more.

FEELING OF FALLING

What is it that drew you to diving?

It's a special way of using your body in space. I also like the feeling of falling. I enjoy jumping off of things. When I haven't done it for a while, I sometimes have anxiety dreams about it. Those go away when I start doing it more. There's just something about engaging your brain in a certain way…

I haven't been able to dive recently because of some injuries, but Stanford has an amazing diving facility. I'm hoping to join the Masters team here when I'm able.

Did you want to be a professional diver?

I competed in diving for several years, but that wasn't the only thing that interested me. I mostly liked to get out and see as much of the world as I could. I had a really great high school biology teacher who showed us some of the cool things that could be discovered in biology. Then, in my freshman year of college, I worked in a lab for the first time. A lot of people have an introspective journey in college, and that was definitely the case for me. Thinking about what I wanted to do in the world, I came to the conclusion that the most important thing I could do was to make new discoveries.

WELL TRAVELED

In whose lab did you work during college?

I worked in a number of labs, actually. I first worked with Teri Melese, a yeast molecular biologist, but I later spent summers in Kenya studying blue monkeys, in Madagascar studying humpback whales, and in a lab studying axon guidance in leeches. After college I worked for two years as a research technician in Debbie Yelon's lab studying heart development in zebrafish. That was the first time I saw the beauty of developmental biology and what you can do with genetics in a really tractable model.

> "The most important thing I could do was to make new discoveries."

You eventually found your scientific home with centriolar biology…

Guided by people in my lab at the time, I went to the University of California, San Francisco as a grad student. In one of our discussion sections for class we read some papers by Wallace Marshall—who was about to join UCSF but hadn't yet arrived—about flagellar and centriolar biology in *Chlamydomonas*. I thought this was so cool that I actually wrote to him ahead of time, asking if I could rotate in his lab when he arrived.

I don't know if you've ever seen a *Chlamydomonas* cell, but they're really cute. They're a single cell with two flagella that they use to phototax toward light sources. They have very robust cell geometry, so everything within the cell is stereotypically positioned. In particular, the centrioles that will give rise to the flagella are specifically anchored at one part of the cell. At that point it wasn't known how centrioles or centrosomes got positioned in any cell type. We used *Chlamydomonas* as a model to ask that question, reasoning that mutations that affect centriolar positioning would also affect phototaxis.

PHOTO COURTESY OF JAMES OCHS

Jessica Feldman

Centrosomes are famous for organizing the microtubule cytoskeleton. But they don't do this in all cell types?

Differentiated cells can actually have microtubules in many different places. For example, in muscle, neurons, and epithelia, we've known for a long time that microtubules are not just organized at the centrosome.

For my postdoc, I wanted to go to a multicellular system where I could study cells in vivo during development in a good genetic model. I ended up working with one of the world's experts on *C. elegans* embryology, Jim Priess. Jim had known for years that centrioles in the *C. elegans* embryonic intestine get specifically positioned at the apical surface. In many epithelial cells, centrioles are positioned at the apical surface in order to build a cilium, but there's no cilium on these cells in *C. elegans*. Why do these centrosomes move to the apical surface if they're not building a cilium?

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

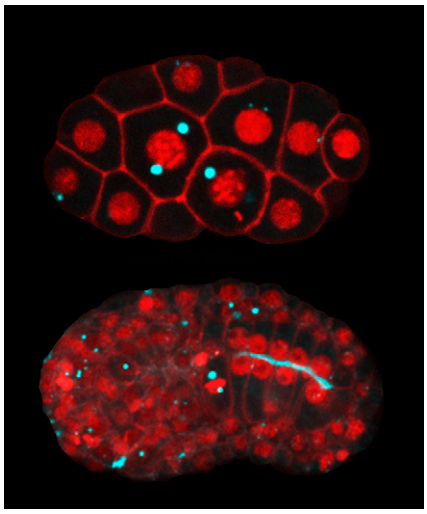

IMAGE COURTESY OF JESSICA FELDMAN

γ-Tubulin (blue) localizes to centrosomes in cycling cells of the early *C. elegans* embryo (top) but to the apical membrane in epithelia during morphogenesis (bottom).

I started doing some imaging to try to understand what was going on.

One great thing about the *C. elegans* embryo is that its development is very stereotypical, so we can image different processes at very specific points. Looking at γ-tubulin, which is a microtubule minus-end protein that is normally found at the centrosome, I was very surprised to see that it was all over the apical surfaces of polarized embryonic intestinal epithelial cells. We found that it actually redistributes from the centrosome to the lateral membrane, and from there to the apical membrane, during polarization of these cells. I started wondering what we really know about microtubule organization, and about how cells regulate microtubule organization at different locations.

ON AGAIN, OFF AGAIN

What proteins control microtubule nucleation at centrosomes or other MTOCs?

γ-Tubulin is part of a complex of conserved proteins that is often found embedded in the PCM, and that promotes microtubule nucleation. γ-Tubulin also seems to help anchor microtubules to the centrosome so that when γ-tubulin is missing, microtubule nucleation is severely crippled, and the remaining microtubules traverse the centrosome in an abnormal way. In *C. elegans* we've observed that γ-tubulin streams in a plume from the PCM to the membrane. XMAP-215, TAC1, and an Aurora-A homologue also move along with γ-tubulin. We think that many of the core proteins that nucleate microtubules are going to be found at both sites, but what we don't know is the identity of the different adaptor proteins that link the minus ends of microtubules to these different sites. That's something we're very interested in looking at right now.

How does γ-tubulin move in a plume?

We would love to know that. One thing we know is that microtubules are probably involved. If we treat the cells with microtubule poisons at a time when we normally see the plume forming, then the plume doesn't form. If we look later on, some microtubules do appear at the apical surface. We don't know if that's just because the nocodazole wears off, or because there's some independent mechanism to build MTOCs.

We also know that there are a bunch of proteins destined for the apical surface, including PAR-3, PAR-6, and E-cadherin, that start out on the lateral membrane and then get moved to the apical membrane. Those proteins appear to move with the γ-tubulin plume, and if we get rid of PAR-3, we don't see the plume.

"I started wondering what we really know about microtubule organization."

What regulates MTOC redistribution?

In *C. elegans* it is very binary; the MTOC is either at the centrosome or at the membrane. In dividing cells the MTOC is at the centrosome, but in polarized epithelial cells it's at the apical membrane. We've recently shown that the centrosomal MTOC is dominant to the apical site. In the *C. elegans* intestine, most of the cells reach the differentiated, polarized state and then stop cycling, while another subset of cells will polarize, but then divide again. We used a laser to destroy the membrane between a dividing cell and an already polarized one, and saw that the centrosome in the polarized epithelial cell was very rapidly reactivated as an MTOC. Lots of γ-tubulin left the membrane and went there instead.

We wanted to understand how the centrosomes gets toggled on and off as an MTOC. There's a relatively simple network of proteins that are required for MTOC function at the centrosome. By marking the different candidates with GFP and photobleaching either the mitotic or interphase cell prior to fusing them together, we could see whether those proteins go to the centrosome, and where they come from. We saw that one protein in particular, the CEP192 homologue SPD-2, goes to the centrosome from the mitotic cell. However, forced expression of SPD-2 doesn't cause centrosomal MTOC activation, so we think that in a mitotic cell, SPD-2 may be modified in some way to allow it to be able to add on to the centrosome. We're now exploring that activation process. We think it's phosphorylation based, and we've shown that a cyclin dependent kinase (probably CDK1) is involved, but there may be other things involved as well.

An interesting aspect to this is that it's been known for a long time that centrosomes have hyperactive MTOC activity in cancer cells, and that this may impact invasive behavior. We're interested to see if we can tune or control MTOC function to affect cancer cell activity or growth.

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Feldman at Venezuela's Mt. Roraima