People & Ideas

Trina Schroer: What's cooking on dynactin

Schroer studies the composition and activity of dynactin.

Dynactin is a multisubunit complex that cooperates with the molecular motor cytoplasmic dynein to drive the minus end–directed movement of organelles and vesicles along microtubules. Dynactin is necessary for dynein's ability to move cargo along microtubules, but, despite 20 years of study, its exact function is unclear and remains controversial.

To Trina Schroer, dynactin's enigmatic nature has been a source of excitement for much of her career. It was while she was a postdoc in Mike Sheetz's laboratory—shortly after having encountered dynein itself (1)—that Schroer first came across a mysterious cytoplasmic activity that promoted dynein motor function (2). Since then, her lab has uncovered details of dynactin's subunit composition (3), its structure (4), and the functions of its different subdomains (5). We called her at her lab at Johns Hopkins University to learn her thoughts on dynactin and to hear what surprises she's preparing for the future.

JUST LIKE COOKING

When did you first become interested in a scientific career?

When I was a child, I was very interested in the domestic arts. I started cooking gourmet meals at a very young age. I liked food, and I liked experimenting in the kitchen. That may have been one of the first indicators that I was going to end up being a good bench scientist. I would try things, and if they didn't work I wanted to know why, and I would try them again. I liked things to be reproducible and to work well. [Laughs]

> "Anything is possible in biology, and you can't get hung up on convention."

I think my first real introduction to science came when I was 10. My uncle, who is a retired physician, gave me a *Time Life* book on viruses, and I was just blown away at the concept of the viral life cycle. By the time I graduated from high school, it was very clear that I was interested in the natural sciences. In fact, on my SAT application form, I wrote that I wanted to get a PhD in biochemistry.

Does science run in your family?

My father is a mathematician, and there are engineers on both sides of my family. My parents certainly both encouraged my interests in science. But my daughter wants nothing to do with science. She's just starting her undergraduate studies, though, so I suppose anything could happen. [Laughs]

Did you actually study biochemistry?

I got my first real taste of biochemistry as an undergraduate at Stanford, in the lab of one of my professors, Gordon Ringold. I loved it! When it came time to apply to graduate schools, Gordon suggested I apply to UCSF. I'd never have thought of applying there on my own; I'd never even heard of it.

You first started working on the problem of cellular transport as a grad student…

Yes. That was really a project of my own making. I'd joined Regis Kelly's lab, which at the time was studying fundamental questions of membrane trafficking and organelle biogenesis, specifically synaptic vesicle biogenesis. But I wanted to develop an in vitro assay to reconstitute vesicle transport along microtubules. I even built a microinjection apparatus—basically from scratch—to inject neurosecretory cells with fluorescently labeled vesicles. But I couldn't get the assay to work; the vesicles clogged the needle. Fortunately, Reg recognized that I needed to try a different model system. When he caught wind of what was going on at Woods Hole, he sent me out there to work for a few weeks with Scott Brady on trying to reconstitute vesicular transport in the squid giant axon.

PHOTO COURTESY OF MARGARET CHARNAS

Trina Schroer

That was my introduction to the world of molecular motors. Ron Vale and Scott Brady both managed to purify kinesin later on that year. It was a very exciting time to enter the field.

FROM SIMMER TO BOIL

You did your postdoc in St. Louis with Mike Sheetz…

I wanted to use in vitro techniques to study the molecular basis of vesicular motion. It was clear to me that Mike Sheetz would be the best person to work with if I wanted to take this approach. I started out looking for a vesicular kinesin receptor in squid giant axons, but I was struggling, in part because squid axons were difficult to get in St. Louis. Then I and some other people in Mike's lab, Sandy Dabora and Eric Steuer, decided to switch to using chick embryo fibroblasts. These cells are abundant and easy to obtain, but the primary type of motility observed in them is the minus end–directed movement of endocytic vesicles. This led us to cytoplasmic dynein, because that is the motor favored by that particular organelle population. I identified an activity in the cytoplasm that caused dynein-associated vesicles to move—this turned out to be dynactin. Of course, at that time, I didn't know what it was; it was just a bunch of bands on a gel. But it got me my job at Johns Hopkins.

Text and Interview by Caitlin Sedwick
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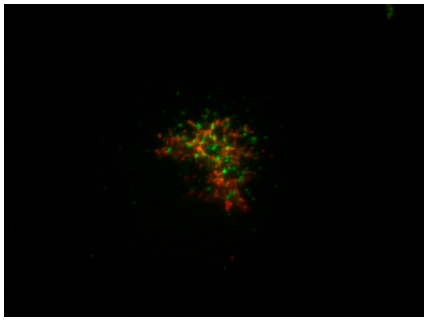

IMAGE COURTESY OF ROB LEVENDOSKY

Schroer's lab investigates dynactin's effects on membrane trafficking by localizing TGN-46 (red) and C1-MPR (green).

What was your first challenge with dynactin?

Early on, I was massively aided by my dear colleague, John Heuser. He's an electron microscopy expert who loves motors, and he was right down the hall in the cell biology department when I was a postdoc at Washington University. Right away we had a big mystery to solve, which was that, when John looked at my dynein activator in the electron microscope, he swore it contained something that looked just like actin filaments. At first I thought the actin was a contaminant, but it soon became clear that it was actually part of the protein complex that makes up the activator.

This was a real head-scratcher at the time because actin-related proteins were completely unknown. Arp2/3 hadn't even been discovered yet. Our mystery protein was the right size to be actin and it looked like actin, so we thought it had to be actin. And yet it reacted poorly with antibodies to actin. Then another group identified Arp3, and, once the notion of actin-related proteins crossed my consciousness, I realized that was probably what we were dealing with. Now we think that this protein, Arp1, provides a structure that favors dynactin interaction with membranes.

I have to say that, since that discovery, I've realized that anything is possible in biology, and you can't get hung up on convention. Let the science tell you what's going on. Do not allow tunnel vision to limit your thinking.

THE RIGHT RECIPE

Does dynactin affect dynein's cargo specificity?

The last year has seen the culmination of a lot of analyses we've done to try to get some sense of what's going on with the proteins that make up the pointed-end complex of dynactin. These proteins aren't present in budding yeast, so they're not required for dynactin's most basic functions. But we think they do help refine dynactin activity, allowing it to govern what dynein does, letting it work in particular places or perform specific functions.

> "It also remains unclear exactly how dynactin influences dynein motor activity."

Dynactin seems to affect both dynein motor activity and membrane association…

Dynactin definitely contributes to dynein binding to membranes. But dynein does have the ability to bind to membranes on its own, and there are a number of other membrane-associated proteins that can bind dynein. The complexity by which dynein interacts with membranes has to be significant, but we're a long way from understanding how its docking and dissociation are regulated.

It also remains unclear exactly how dynactin influences dynein motor activity. My lab has shown that dynactin and dynein, together, are sufficient to drive vesicle motility in vitro. But the dynein pathway is very complicated. Besides dynein and dynactin, it includes other proteins called LIS1 and NUDEL. Genetically these components all seem to be of equal importance to dynein, even though they're not required in vitro for dynein motor activity.

This apparent contradiction could be due to the fact that, because the large, multisubunit dynein molecule is difficult to work with, most in vitro assays use a very simplified version of dynein. To reconcile these disparate findings and fully understand dynactin's role in the dynein machine, we need to perform more sophisticated assays that require the entire dynein molecule. The problem is that all these proteins are notoriously hard to obtain and work with. But my lab is good at purifying native dynein and dynactin, so we're collaborating with other groups to look at questions like these. For example, we've recently begun collaborating with Gabe Lander at Scripps to solve the structure of dynactin using cryo-EM, hoping to gain a better understanding of the dynein–dynactin interaction.

Do you have any advice for beginning researchers?

As a member of several journals' editorial boards, I can assure you that we work very hard to protect the integrity of the literature. But, despite the best efforts of editors and reviewers, there is still a large amount of published material that's incorrect, overinterpreted, or not reproducible. So, I always remind people in my group that it's important to evaluate the data critically and draw your own conclusions. Sometimes the only way to do this is by repeating experiments that have already been published. It may take one longer to get to an end story, but an important goal is to publish reliable, go-to papers that people can be confident in.

1. Schroer, T.A., E.R. Steuer, and M.P. Sheetz. 1989. *Cell*. 56:937–946.
2. Gill, S.R., et al. 1991. *J. Cell Biol.* 115:1639–1650.
3. Eckley, D.M., et al. 1999. *J. Cell Biol.* 147:307–320.
4. Schafer, D.A., et al. 1994. *J. Cell Biol.* 126:403–412.
5. Yeh, T.-Y., et al. 2012. *Mol. Biol. Cell*. 23:3827–3837.

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A successful culinary experiment yields a delicious Bûche de Noël.