Anna Akhmanova: Great tips on microtubules

Akhmanova studies microtubule plus end–binding proteins and proteins that interact with microtubule motors.

The microtubule cytoskeleton not only gives cells their shape but also sets up and maintains cell polarity. For example, microtubule-based motors help establish polarity by delivering certain cargoes to specific sites, and microtubule plus end–tracking proteins (+TIPs) help guide the growth and connection of microtubules to the cell cortex and other cellular structures. Anna Akhmanova is intent on working out how +TIPs interact with microtubules and with each other (1–3) and how motor proteins bind their cargoes (4, 5), with the aim of better understanding how cell polarity is generated.

Born and raised in Moscow in a family of scientists, Akhmanova says she was interested in nature even as a child, so her decision to pursue a research career was a natural one. Things have continued to progress since then, and we reached her at her new laboratory at Utrecht University to talk about how she's building her career.

PROMISING START

What made you decide to pursue a career in research?

All of my family are scientists. My grandmother was a professor in English and linguistics, and my father was a professor in physics. Both my mother and brother hold PhDs in physics. So, I was surrounded by scientists from my early childhood, and a career in science was a very natural choice for me.

What did you focus on in school?

I went to school at Moscow State University, where I studied biology. At that time, higher education in Russia was not divided into bachelor's and master's degrees like it is in Western universities. There was just a single, five-year-long program, and at the end of it you received your master's degree.

For the first two years we studied all the major biology subjects, like botany and zoology, but also biochemistry, molecular biology, and cell biology. Then, like everyone else, my fifth year was dedicated to doing a big research project. I studied halophilic archaebacteria in Alexander Mankin's laboratory. I learned most of the molecular biology I know from him.

RESTRUCTURING

Why did you decide to leave Russia to pursue your PhD?

When I finished my master's in 1989, things in Russia were changing drastically; this was during the time of perestroika. Before perestroika, the university and research system in Russia was doing okay. But then, in a very short period of time, it pretty much dissolved, so a lot of people went to work abroad. I tried to become a PhD student in Russia, but the salaries were very low, there was absolutely no funding to do research, and the country as a whole was experiencing problems that were far more serious. So, when I got an opportunity to go to the Netherlands, I decided to go there to do my PhD.

> "CLASPs have the capacity to make the microtubule system asymmetric."

Was that a difficult transition for you?

It was difficult. I had a very small child at that time, and I was trying to do my PhD and adapt to a foreign country. But I think that when you're young these types of transitions are easier. I do not think I would be able to cope with that kind of challenge now, but at the time it was okay, and it was all very worthwhile. My daughter is a real driving force in my life outside of the lab.

Scientifically, at that time, I was interested in gene regulation, so I joined Wolfgang Hennig's lab at the University of Nijmegen and tried to obtain mutants of different histone genes. For various reasons, the project did not go very well, so, although these years were very formative for me, maybe they were less good in terms of having scientific success.

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Anna Akhmanova

But you kept on…

I actually did two postdocs. For the first one I stayed on at the University of Nijmegen and worked on several anaerobic organisms in the Department of Microbiology. For my second postdoc, I went to the Erasmus University of Rotterdam, to the Department of Cell Biology led by Frank Grosveld, because I wanted to work on transcription and gene regulation again. I joined Niels Galjart's lab to work on a particular transcription factor, and for part of that project I had to perform a yeast two-hybrid screen with that protein. While I was setting that up, the other person in the lab, Casper Hoogenraad, asked me if I would help him screen a protein that he was working on, a microtubule-binding protein called CLIP-115. I did, and we picked up some clones: one of them was a very poorly studied protein, which we called CLASP, for CLIP-associating protein. The other was a homologue of a well-studied fly protein called Bicaudal-D. These two hits defined my career.

Text and Interview by Caitlin Sedwick
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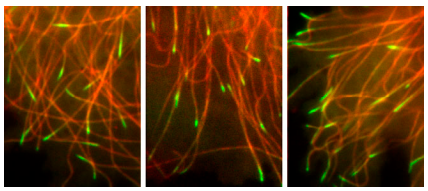

IMAGE COURTESY OF DR. ILYA GRIGORIEV

Microtubule plus end–binding proteins (green) decorate the growing ends of microtubules (red).

WELL ESTABLISHED

What did CLASP turn out to do?

We now know that CLASPs are a family of proteins that can bind to microtubules directly but that also can interact with the microtubule plus end–binding protein EB1. Probably both interactions are important to allow CLASPs to bind microtubules and to allow CLASPs to become enriched at the growing microtubule plus end, where they may help stabilize microtubules. But CLASPs also have a domain that binds other proteins, including one called LL5b, which is a lipid-binding protein found at the plasma membrane and predominantly localized at a cell's leading edge.

The reason why we originally became interested in CLASPs is because, unlike many other microtubule-binding proteins, CLASPs are preferentially enriched at the leading edge of migrating cells. That is partly due to interactions with LL5b but also because CLASPs are strongly regulated by a kinase called GSK-3β. GSK-3β is present throughout the cell, and, when active, it suppresses the binding of CLASPs to microtubules. However, this kinase is inactive at the leading edge, so CLASPs bind best to microtubule plus ends at the leading edge. In this way, CLASPs have the capacity to make the microtubule system asymmetric. This is important for cellular polarity and phenomena that depend on it, such as cell migration and polarized secretion.

What about the other hit in your screen?

When we first found Bicaudal-D, we were just really curious what the protein was doing, and that led us into studying how motors attach to different cargo. We've been studying cytoplasmic dynein, which is a very large, ubiquitous, minus end–directed microtubule-based motor. For quite a few years we've been trying to understand how Bicaudal-D binds to dynein and how it attaches to its vesicular cargo. We've found that Bicaudal-D binds to dynein via its N-terminal region and binds to the small GTPase Rab6 via its C-terminal region. Rab6 binds to the vesicular membrane, completing the bridge between dynein and its cargo vesicles.

Bicaudal-D is an interesting molecule because, if you just put it into cells, it actually doesn't do much. However, if you take the N-terminal part of the molecule and attach it to almost any cargo, it induces very rapid and efficient transport by cytoplasmic dynein. Also, the C-terminal part acts as a dominant-negative for Bicaudal-D function: it not only blocks binding of the wild-type protein to Rab6-positive cargo vesicles but also blocks Bicaudal-D binding to the nuclear envelope during the G2 phase of the cell cycle, where it normally helps to position the nucleus and centrosomes relative to each other.

We think that Bicaudal-D as a whole is self-inhibited—it adopts a folded conformation that prevents it from interacting with other proteins. Bicaudal-D is not the only microtubule-interacting protein that behaves this way; a couple of years back we published a paper about a microtubule plus end–binding protein, CLIP-170, that is also self-inhibited. I'm interested in how these types of molecules are self-inhibited and how they're activated.

Where is this work taking you now?

I'm collaborating quite strongly with Casper's laboratory, and in January we both moved our labs to Utrecht University, where we are running the Division of Cell Biology together. I am happy because our labs are now right next door to each other, and we can preserve our collaboration.

I've also recently become very interested in structural studies. That's partly because a couple years back I started to collaborate very intensively with Michel Steinmetz's lab in Switzerland on the structure of +TIP complexes. I think that in the future it will be important to understand what Bicaudal-D's interactions with other proteins look like at the molecular level: what the structure is and how the structure translates into function.

> "The diversity of the plus end–binding proteins is much bigger than we previously thought."

We are also working to identify more microtubule plus end–binding proteins, particularly proteins that interact with EB1 and its relatives EB2 and EB3. What we're currently discovering is that the diversity of the plus end–binding proteins is much bigger than we previously thought. It will be exciting to get our hands on this whole zoo of proteins.

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Akhmanova's daughter (right) orchestrates a zipline mother–daughter adventure.