Alexey Khodjakov: Diving into the mitotic apparatus

Khodjakov uses cutting-edge microscopy to study how the mitotic spindle is organized.

Situated at either end of the animal mitotic spindle, centrosomes are one of the most prominent and visually striking features of the mitotic apparatus. But what, exactly, is their function in cell division? And how is the mitotic spindle organized in dividing cells?

Alexey Khodjakov has been grappling with such questions since he was a graduate student in Russia. Using advanced microscopy techniques including laser ablation (1) and 3D particle tracking (2), Khodjakov has delved deeply into the organization of the mitotic apparatus (3, 4) and its various components (5), making big splashes in the field along the way. We called him at his laboratory at the Wadsworth Institute in Albany, New York, to learn more about his personal and professional explorations.

ON THE SURFACE

Where did you grow up?

I grew up in Russia. In Moscow, to be exact. I was supposed to be an engineer like my entire family—my father had a PhD in engineering, and my mother and sister were both engineers. But I am a rebel. [Laughs] When I announced that I was going into biology, my mom was convinced that I would die hungry. Somehow, though, I just connected with biology in secondary school. It was interesting. It wasn't the complexity of different life forms but looking at cells and trying to understand how they worked that fascinated me.

To make a long story short, after secondary school, I applied to the biology program at Moscow State University. The entrance exams were very difficult, and I didn't get in on my first attempt, but I did on my second try, and I really enjoyed my studies. I did my PhD there, as well.

What did you work on in graduate school?

In a way, I was working on pretty much what I work on now. I was obsessed with mitosis because Tim Mitchison and Marc Kirschner had just published their review on dynamic instability and their search-and-capture hypothesis, so I looked for a mentor who was working on mitosis. One of the questions I worked on was whether an animal cell without centrosomes can divide and progress through the cell cycle. Unfortunately, we couldn't do it with the technology of the time, but that ultimately became a subject that I have studied in my own laboratory.

DIVE PLAN

Did you have any plans to study abroad?

No. I loved being at Moscow State University and expected to spend my entire career there. But in 1990, right before I defended my PhD, there was a conference in St. Petersburg. For the Americans at the conference, it was an opportunity to go behind the Iron Curtain and see what was going on in the Soviet Union. For me, it was an opportunity to meet all the famous Western scientists I idolized. That's where I met Conly Rieder and realized that I really wanted to work with him.

I didn't plan to go to the States, though; I wasn't even considering doing a post-doc because they weren't common in Russia. But in the wake of this conference, Ryoko Kuriyama contacted my mentor in Russia, who was the department chair, to ask if he knew someone who could be a post-doc. It was a great opportunity, and about six months later I was in Minnesota. This was right after Mikhail Gorbachev's famous visit to the city. Believe it or not, people were pointing at my wife and me in the streets.

Did you continue your studies on centrosomes with Kuriyama?

Yes, but using different techniques. When I came to Ryoko's laboratory, I was mostly doing molecular biology, looking for potential centrosome components. After two years in Ryoko's laboratory, I returned to Russia and got a position at Moscow State University and a secondary position at the Institute for Molecular Biology and had absolutely no intention of returning to the United States. But in 1994 there was a second coup in Russia and yet another financial crisis, so it became difficult to isolate proteins, clone DNA, and do other things like that. My wife and I decided to move for a year or two at most.

PHOTO COURTESY OF IRINA TIKHONENKO AND MICHAEL KOONCE

Alexey Khodjakov

> "Centrosomes are facilitators. They help in a lot of different aspects of a cell's life."

I wrote two letters, one to Conly and one to Kip Sluder, and asked if they had a senior post-doc position for a year or two. Conly immediately replied and said, "Yeah, come on over. We'll have fun," so we moved to Albany. Some time later, my wife and I looked at each other and said, "It's probably time to face the reality." Our son, who was brought to this country at the age of two, back to Russia at the age of four, then to Albany when he was six, was already in middle school. We realized that we were staying.

With Conly, you got back to working on centrosomes…

Text and Interview by Caitlin Sedwick
csedwick@gmail.com

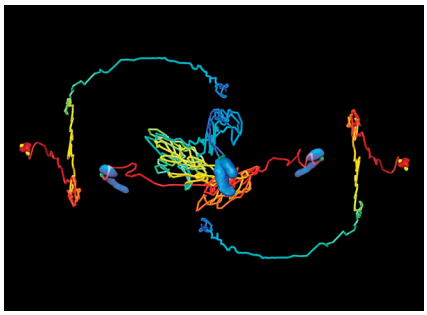

IMAGE COURTESY OF VAIENTIN MAGIDSON AND CHRISTOPHER O'CONNELL

3D trajectories show the movements of individual chromosomes from the onset of mitosis on through to anaphase.

Conly really taught me how to watch cells and how to understand them. At the same time, we started to use lasers to slice and dice chromosomes, kill individual kinetochores, and slice in between kinetochores to see how chromosome movements would be affected. Then I attended the 1997 ASCB meeting, and the whole conference was full of presentations on GFP. Because I was playing with the laser, I immediately realized that, if we labeled the centrosome with GFP and then used a laser to ablate it, then I could finally do what I had tried to do in graduate school: create a cell that would completely lack centrosomes. I came to Conly and said, "Hey, you mind if I do this GFP thing?"

A DEEPER LOOK

What have you since learned about centrosomes?

It turns out that cells can do everything without centrosomes except form cilia. We showed that you can remove the centrosome right before mitosis and the spindle still forms, although the fidelity of chromosome segregation is not as high as under normal conditions. In that study, we observed that cells that are born without centrosomes fail to progress through G1, but later we discovered that was because the cells were arresting due to stress. Cells lacking centrosomes are very sensitive to external insults, so, when we used milder conditions, all of a sudden cells started to progress through the entire cell cycle. Now I think we realize that centrosomes are facilitators. They help in a lot of different aspects of a cell's life—not just cell division—but they're not absolutely necessary.

But having extra centrosomes is bad, so why do we still have them?

That's a very good question. I wish I knew the exact answer, but that's exactly what we're trying to understand. There must be a reason. There are probably multiple reasons, because not all cells have centrosomes. For example, plant cells lack them completely. They do everything that animal cells do, but somehow animals rely on the presence of centrosomes. There must be some selective pressure to maintain them.

> "I believe that the shape and the position of kinetochores on the centromere changes throughout the cell cycle."

You've also studied how kinetochores attach to the spindle…

What we've tried to address in our recent work is what a typical chromosome does. Previously, it had been difficult to visualize the movements of individual chromosomes during mitosis. Using photoactivation, we were able to reconstruct what chromosomes in the middle of the spindle do when the spindle assembles. We showed that, initially, the very first movement is toward the surface of the nascent spindle so that the chromosomes become positioned in a donut around the center part of the forming spindle. There, they form lateral attachments to spindle microtubules that are later converted to minus-end attachments.

What is the focus of your laboratory now?

We're looking at the constraints that come from the shapes of kinetochores and from the positions of microtubules, microtubule-organizing centers, and kinetochores. Kinetochores are two tiny dots—in humans, typically only 200 nanometers. And yet we see that chromosomes manage to jump on microtubules almost instantaneously after nuclear breakdown. That implies that the interaction is somehow favorable. But later on, it's important to ensure that the kinetochore is attached to only one pole and that the sister kinetochore is attached to the opposite spindle pole. I believe that the shape and the position of kinetochores on the centromere changes throughout the cell cycle to facilitate these changes in behavior. We are just beginning to describe these changes.

I'm an unbelievably lucky person because, for whatever reason, I have had some fantastic people come through my laboratory. Much of my research wouldn't be possible if I didn't have the right people with the drive to see the work through.

Is your laboratory also your major hobby?

Actually, I dive every chance I get. All kinds of diving: tropical, cold-water, wreck diving, wherever I can. My wife dives, as does my next-door neighbor in the laboratory, Mike Koonce. If we're not in the laboratory, chances are we're diving. Every Saturday we wake up at six o'clock, then either I drive to his house or he comes and picks me up, and we go to a particular marble quarry in Vermont. The water temperature there is 41°F year 'round, so without a dry suit it'd be…

A little cold?

No, it's "a little cold" when you wear a dry suit! [Laughs]

1. Khodjakov, A., et al. 2000. *Curr. Biol.* 10:59–67.
2. Magidson, V., et al. 2011. *Cell*. 146:555–567.
3. Kapoor, T., et al. 2006. *Science*. 311:388–391.
4. O'Connell, C.B., et al. 2009. *J. Cell Biol.* 187:43–51.
5. Loncarek, J., et al. 2008. *Nat. Cell Biol.* 10:322–328.

PHOTO COURTESY OF ALEXEY KHODJAKOV

Khodjakov diving a wreck in Truk Lagoon, Micronesia.