Guangshuo Ou: New perspectives on Q cell biology

Ou studies asymmetric stem cell division and differentiation in *C. elegans* Q neuroblasts.

Classic experiments in *C. elegans* showed that a neuroblast stem cell, called the Q cell, undergoes sequential asymmetric cell divisions that result in daughter cells with dramatically different cell fates. The daughters of the first division migrate to different locations in the animal where they divide again. A further round of division generates a small daughter cell that immediately dies by apoptosis and a large daughter cell that migrates and differentiates.

For Guangshuo Ou, the *C. elegans* Q cell lineage is the perfect system in which to study the mechanisms of asymmetric cell division (1) and the processes that drive cell migration (2), differentiation (3), and apoptosis (4). The classic studies had stopped short of probing Q neuroblast biology in depth, but Ou's application of modern microscopy and live-cell imaging has shed new light on the subject. We called him at his lab at China's Tsinghua University to learn about the new technologies (5) he's bringing to *C. elegans* stem cell biology.

SLEEPLESS NIGHTS

Who in your early life influenced your decision to go into research?

My dad is a scientist who works on global positioning systems, and he's an investigator at the Chinese Academy of Sciences. So it's probably not surprising I wanted to be a scientist early on. He promoted my sense of curiosity about the world and encouraged me to try various things. Also, he's a tiger dad—you know, like tiger moms—so he was very strict with me about my studies.

As a result I did pretty well in middle school. My scores were good enough to get me into one of the best high schools in Wuhan, where I grew up. There, I was selected into a special class where students trained to compete in the International Olympiad for mathematics, physics, or chemistry. However, I quickly realized this was a mistake for me. Some of my classmates were real geniuses, and I doubted whether I was smart enough. I became anxious, couldn't focus on anything, and couldn't sleep. But during these sleepless nights, I figured out some things that helped me out and that still influence me today.

First, I should not compare myself to my classmates. I should just try to make progress every day, and gradually I would be able to solve even very hard problems. Second, I decided I should focus on biology, because I wanted to be a scientist and I felt I could be better in this subject than in other disciplines.

And you came to the US for your PhD…

I had done a master's degree on actin cytoskeletal dynamics in plant cells with Ming Yuan at China Agricultural University. For my PhD, I joined Jon Scholey's lab because I wanted to work in a model system where I could combine live-cell imaging with genetics, and Jon's lab was using this approach to study the intraflagellar transport (IFT) motors that assemble sensory cilia in *C. elegans*.

> "Bringing an advanced technique to an old problem can provide new discoveries."

I first chose to work on identifying binding partners of IFT motors. But after half a year I still didn't have any very exciting or interesting results, so Jon suggested that I use live-cell imaging to visualize IFT. I did not understand why he was suggesting this because, four years earlier, Jon's lab had published two papers where they used time-lapse microscopy to study intraflagellar transport. He explained that I should use the new spinning-disc confocal microscope to reexamine the subject. I just didn't agree. We debated whether I should do this experiment for an entire week. The experiment is actually very straightforward and easy to do, but I was stubborn.

PHOTO COURTESY OF GUANGSHUO OU

Guangshuo Ou

Finally I decided I should just do the experiment, and immediately I found something totally unexpected! Intraflagellar transport was first discovered in *Chlamydomonas*, where materials for constructing the cilia are transported to the tip of the cilium by a single type of kinesin-2 motor. But in *C. elegans* I found that intraflagellar transport involves sequential anterograde transport pathways driven by two distinct types of kinesin-2 motors. Now, similar motors are being studied in cilia of several organisms. It taught me a big lesson: bringing an advanced technique to an old problem can provide new discoveries.

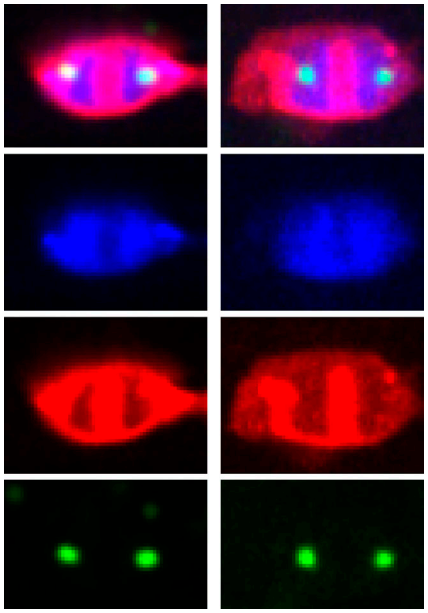
OLD PROBLEM, NEW APPROACH

This lesson was useful in your postdoc…

I wanted to use live imaging to study cell biological problems in living organisms, and I also wanted to work in Ron Vale's lab. So I studied his lab's web page carefully and saw that he had a project using live-cell imaging during planarian regeneration. I thought, "That's a perfect match!"

Ron offered me a position to come work on this project. We decided I should start by making a transgenic planarian that expresses fluorescent markers in its stem cells. For about 16 months, I tried everything I could think of to make a transgenic planarian but with no success, so I decided to try studying how the first transgenic animals were constructed. One day I read the classic paper by John Sulston and Bob Horvitz that describes how they used Nomarski optics to monitor

Text and Interview by Caitlin Sedwick
csedwick@gmail.com



IMAGES COURTESY OF GUANGSHUO OU

Q.a (left) and Q.p (right) neuroblasts divide asymmetrically to generate daughter cells with dramatically different cell fates.

the division, migration, and apoptosis of *C. elegans* Q neuroblasts. I thought, "This is so cool! I can use spinning-disc confocal and long-term fluorescence imaging to study the behavior of these stem cells and maybe make some unexpected observations."

I got one of the classic GFP-tagged strains from Cynthia Kenyon's lab next door, which used to work on Q cells. But when I put it on the microscope I saw the marker was expressed in hundreds of cells, and I could not identify the Q cell. So I thought, "Oh, that's fine. If I make a movie and see a cell that divides and then migrates, that's probably a Q cell." Looking back, this seems like a totally backwards approach. [*Laughs*]

I set up the movie, and indeed I found one cell that underwent asymmetric cell division. The larger daughter cell began long-distance migration while the small one died. I had found the Q neuroblast. I was so excited! My first paper in Ron's lab was about Q cell migration, and the second was about Q cell asymmetric cell division.

Was it always your plan to return to China after your postdoc?

When I first started my postdoc, I didn't plan to return. For six years I was really excited about living in the US, but in my eighth year all of a sudden I started feeling quite homesick. I discussed it with my wife, and we decided we should go back to China. I just felt it was time to come back.

Also, around that time, research funding in China was going up at approximately 20% per year. There were many good job opportunities there and good start-up funding, so I could build the same microscope system I had used in Ron Vale's lab.

CHALLENGE, ACCEPTED

In your own lab you first studied the fate of the apoptosing daughter cell…

That's right. The big question we wanted to answer was what the function of autophagy is during apoptosis. In collaboration with Xiaochen Wang, we used autophagy mutants to examine whether they showed any apoptotic defects for this apoptosing daughter cell. Interestingly, we found that autophagy genes probably do not do anything for the dying cell. Instead, they act in a neighboring epithelial cell, called the hyp7 cell, which engulfs and degrades the apoptotic cell corpse.

> "I had found the Q neuroblast. I was so excited!"

The next thing we looked at was the Q cell midbody, which is a little bundle of proteins and microtubules that forms between dividing daughter cells. I wondered if the midbody might contribute to the cell fate of one of the daughter cells. If it does, then it would probably be inherited by the large daughter cell. If it is just trash, it might go with the smaller daughter cell, which will undergo apoptosis. However, what I saw was that the midbody was often released into the environment, where it is taken up by the hyp7 cell. We showed that both midbodies and the dying Q cell daughter stimulate their uptake by the hyp7 cell through exposure of phosphatidylserine. We've found that animals with mutations in the uptake pathways have no cell division phenotype in Q cell lineages. So we suspect that midbodies are just some kind of debris from cell division and that they do not influence cell fate.

What challenges do you face now?

Recently, we have been working to develop conditional knockouts in *C. elegans* to use in studying the molecular mechanisms of Q neuroblast development. This has been challenging because the application of Cre-Lox is difficult in this organism and RNAi technologies are inefficient in the *C. elegans* nervous system.

Fortunately, about two years ago, we were discussing this problem in a lab meeting when we had a magic moment. An undergrad in my lab, Ze Cheng, told me about an artificial DNA nuclease called TALEN that can recognize and cut specific DNA sequences. We discussed ways we could use this to create conditional knockout animals, and then he just set out and did all of these experiments by himself. After one month he told me it was working! Next we started working with another endonuclease system called CRISPR-Cas9, and we succeeded with that as well.

There are a thousand evolutionarily conserved genes that, when mutated, are embryonic lethal in *C. elegans*. Because of this, their function in Q neuroblasts has never been examined. Right now we're picking about 100 genes that are embryonic lethal across many different organisms, and we're constructing conditional knockout animals to see if we can find any novel phenotypes in Q neuroblasts.

1. Ou, G., et al. 2010. *Science*. 330:677–680.
2. Ou, G., and R.D. Vale. 2009. *J. Cell Biol.* 185:77–85.
3. Feng, G., et al. 2013. *Development*. 140:3838–3847.
4. Li, W., et al. 2012. *J. Cell Biol.* 197:27–35.
5. Cheng, Z., et al. 2013. *Nat. Biotechnol.* 31:934–937.

PHOTO COURTESY OF GENG WANG

Ou's lab is bringing new technologies to bear on an old problem.