Yukiko Yamashita: The centrosomes get there first

Yamashita studies how germline stem cells orient their asymmetric cell divisions.

The asymmetric division of stem cells gives rise to two daughter cells with different fates: one that retains stem cell identity and another that is destined to follow a particular developmental path. To guide the differential distribution of cell components, asymmetric division relies on the specific orientation of the dividing cell's microtubule-based spindle apparatus. How this event occurs, and how it impacts stem cell behavior, are questions that fascinate Yukiko Yamashita, professor of cell and developmental biology and Howard Hughes Medical Institute investigator at the University of Michigan.

Yamashita has always enjoyed observing nature in action—first in her mother's garden and later through a microscope while studying cell biology at Kyoto University (1, 2). She put her observational skills to good use as a postdoc in Margaret Fuller's lab at Stanford, where she showed that, in *Drosophila* germline stem cells, spindle orientation is determined even before the spindle forms via precise placement of the centrosomes that will later anchor the opposing poles of the spindle apparatus (3). She's since devoted her career to studying the significance (4, 5) and regulation of this phenomenon (6). We called her to learn more about it and to hear how her career has grown.

OBSERVING NATURE

I understand you grew up in Japan…

Yes. My hometown is a small town near Kobe. Actually, it's a small town by Japanese standards, but in Japan all places are connected to the big city. It's not like it is in America, where you can leave the borders of a town and find yourself in the middle of nowhere.

My mother had a bachelor's degree in pharmacology and worked for a pharmaceutical company until she had me. Then she became a pharmacist and has worked for a hospital ever since. My father works in the patent office, but his real passion is physics.

Who were your role models, growing up?

My father gave me lots of interesting ideas, and he taught me early on about the value of creative thought. These days, I'm becoming more and more aware that my creativity is due to him. But he's rather eccentric, so I don't think I would call him my role model. I guess that as a child I never tried to see myself in someone else. I never wanted to be like some specific person. And for a long time I didn't know what I would like to do as an occupation.

I liked science, but I never thought that could be a job. Probably only after I went to college and went through grad school did I think this would be my occupation.

Why did you leave Japan for your postdoc?

There were a few reasons for that. One was that, after completing my PhD, I felt my passion toward science beginning to wear off. Around that time I got married, and my husband saw me getting stuck and thought it would be a good idea to try changing our environment.

> "The two centrosomes split apart even before they establish a spindle."

While we were trying to figure out how to do that, I took a short postdoc in a lab nearby and felt my enthusiasm beginning to recover. But I still wanted to try for a big change, because I felt it would be very difficult for me to succeed as a scientist in Japan. At that time in Japan, women researchers stood out a lot, and many people would question your choice to pursue this career. Things are changing today and the environment for women is improving, but at the time I struggled with it a lot.

NEW ENVIRONMENT

Why did you choose to go to Stanford?

My husband first found a lab in Stanford, so that's why I started to look at labs there.

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Yukiko Yamashita

That's how I found Margaret Fuller, and my very first week in Minx's lab I felt, finally, like a weight was lifted. She showed me that you can be yourself and that there's nothing unusual or unexpected about being a female scientist. Minx was probably my first role model. For the first time in my life, I thought, "Oh, I can be like her."

How were you introduced to the subject you worked on as a postdoc?

When I arrived at her lab, Minx had several ideas for projects I could work on. The one that appealed most to me was looking at spindle orientation in the stem cells of fly testes. This had never really been done before, so the first thing we did was to express tubulin-GFP using a germline-specific driver. We saw right away that, in the testes, germline stem cells are arrayed around a hub cell and that the stem cells' spindles are all oriented perpendicular to the hub cell. That wasn't really a surprise because it was already known that cells orient their spindles this way in the female germline stem cell niche. In fact, we'd expected that we would just confirm this and that then we could use this as the basis to build a new experimental system.

But then I noticed something that we hadn't expected: the two centrosomes split apart even before they establish a spindle

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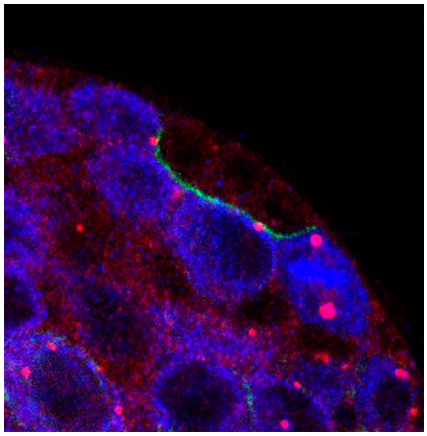

IMAGE COURTESY OF YUKIKO YAMASHITA

The centrosomes (red) of male germline stem cells (blue) orient perpendicular to a hub cell (interface in green) at the apical tip of the testes. The rightmost cell is dividing.

and migrate toward the opposite sides of the cell. Any textbook will tell you that centrosome separation happens at the transition from G2 phase to mitosis, but in our male germline cells the split happens way before mitotic commitment. And I thought, "Wow, this is interesting and weird," so I started puzzling it out. Two years later we had our first paper. Since then, it's been confirmed that this early centrosome migration happens in lots of other kinds of cells, including *Drosophila* female germline stem cells and neuroblasts. It might also occur in mouse neuronal stem cells. So maybe it's not so weird after all.

This centrosome separation occurs in a very characteristic fashion…

We know that the mother centrosome always remains close to the hub cell while the daughter centrosome migrates away. So, what drives daughter centrosome movement? Our recent data suggest that mother and daughter centrosomes are not just structurally different but might also be functionally different. For example, we have a mutant animal where the mother centrosome but not the daughter centrosome elongates abnormally within the stem cell. Perhaps we will find a molecule that only goes to the mother centrosome or the daughter centrosome and then contributes to distinguishing the two from each other.

NEW SIGHTS

Is this process efficient in all germline stem cells?

In young flies it is. But we noticed that, if you look at older flies, centrosomes are not correctly positioned as often as they are in young flies. As they age, the frequency of centrosome misorientation keeps going up. Another curious thing we saw is that, even though centrosome misorientation increases with age, spindle misorientation doesn't increase at all. What could explain this discrepancy? I wondered whether there might be a checkpoint that helps the stem cell make sure its spindle is oriented correctly before it starts dividing. If there is a checkpoint that arrests or delays the cell cycle, then fewer stem cells would divide in older animals, and fewer sperm would be made. This does in fact happen in older flies, so we proposed that this might explain the defects in sperm production seen with aging.

> "The stem cell has a very peculiar behavior for the X and Y chromosomes."

Tell me more about how the checkpoint works.

So far, we have identified several components of this checkpoint. Our recent data suggest this is really a stem cell–specific checkpoint. Other cell types don't have it, and it operates at the G2/M transition.

Right before entering mitosis, the mother centrosome arrives at a docking site near where the germline stem cell contacts the hub cell. Our data suggest that this docking site is very tiny, and it is normally occupied by a structure called the spectrosome. We think the spectrosome sends a "wait" signal, but, once the mother centrosome gets there, it displaces the spectrosome and triggers a signal that allows mitosis to begin. We'd like to know what proteins are involved in regulating the "wait" and "start" signals.

What are other hot topics in your lab?

In the stem cell field, there's an idea called the immortal strand hypothesis. It proposes that, after DNA duplication in S phase, the copied strand is less reliable than the template strand. Stem cells divide many times, so probably stem cells want to keep the original and give copies to their daughters. When we published our mother centrosome work, some people theorized that the mother centrosome might help preserve the immortal strand. That put us in an uncomfortable situation because we hadn't tested the immortal strand hypothesis, but our work was being cited as a kind of evidence for it. So then my grad student wanted to test this idea in our system, and she originally completely ruled it out.

She showed that sister chromatids are not really distinguished between original versus new copy, if you look at the entire genome. The chromosomes seem to be totally randomly segregated. We published this observation. But then, in following up that work, she found that the stem cell has a very peculiar behavior for the X and Y chromosomes. The stem cell can distinguish which chromatid is which and then segregate them differently so that the new copy usually ends up in the daughter cell. This happens only for sex chromosomes, not autosomes.

We don't know how or why this happens. But as a scientist, I couldn't be in a better position. It's great to have so many questions in front of me.

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Yamashita's lab members gathered for a group photo.