Elizabeth Chen: Fusing cells press closer

Chen studies cell–cell fusion in *Drosophila* myoblasts.

Most cells in the body are solo entities, self-contained within a cell membrane. But there are certain cases in which cells meld together. Skeletal muscle cells, for example, are multinucleate syncytia that form through the process of cell–cell fusion. Osteoclasts, a type of cell involved in bone remodeling, also fuse together to promote better bone resorption. And you would not be reading this article were it not for a cell–cell fusion event at the very beginning of your life: the one that takes place between sperm and egg.

In her lab at Johns Hopkins University, Elizabeth Chen studies cell–cell fusion in *Drosophila* myoblasts, aiming to uncover the fundamentals of the process (1). Leveraging the organism's tractable genetics together with advanced imaging techniques (2), her group has demonstrated that myoblast fusion is an actin-dependent process (3, 4) wherein one cell pushes a protrusion deep into its prospective fusion partner (2, 5). Now her lab is taking an even closer look at the determinants of cell–cell fusion, as we learned when we spoke with her recently.

DRIVING FORCES

I understand you're originally from China…

I grew up in a northeastern province in China in a city called Changchun. Both my parents were teachers in a hydroelectric institute. So my family was very intellectual. I read a lot of books when I was little, and I was always curious about nature. I just loved being outdoors. I still do actually.

I think I always wanted to be a scientist. [Laughs] Looking back, I'm not certain whether it was because of the atmosphere at that time in China or because of my love of nature. At the time science was considered a really important career to pursue. In school we were always told that scientists are one of the driving forces of humanity and civilization. I agree!

I went to college at Peking University to study biochemistry, but I was a little disappointed. For example, our cell biology textbook at that time was outdated and printed on a hundred pages of 6-inch by 8-inch paper. I just felt like I didn't get to learn a lot in school, and that's actually one of the reasons why I came to the States. Fortunately, both of my parents had left home at a young age to develop their careers in a different place, so they were pretty supportive of me going to the States to further my education.

Was there something in particular that you wanted to study?

I was accepted to the Department of Chemistry and Biochemistry at UCLA, and I was so excited to be there. I took a wide range of courses to expose myself to different areas of biology, and I joined a lab in my second year, working on transcription initiation in bacteria. But then I fell in love with developmental biology and transferred to Stanford to do my PhD in developmental biology. I was really impressed by *Drosophila* genetics, its rich history and the beautiful little markers that could tell us so much. Also, I discovered that I love drawing up genetic schemes. [Laughs] I joined Bruce Baker's lab because he's a great *Drosophila* geneticist. He taught me all kinds of cool genetic tricks that I've used over the years.

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FOUNDING SYSTEM

What drove your choice of a postdoctoral lab?

As a graduate student I was studying the imaginal disc that gives rise to sexually dimorphic structures. No one in the lab—and very few people in the world—were working on this imaginal disc. It was a good experience because it forced me to become independent very quickly. I laid the groundwork for studying a new system, but I didn't get to build upon it. So for my postdoc I wanted to find a system where some of the basic information was known and I could expand upon it.

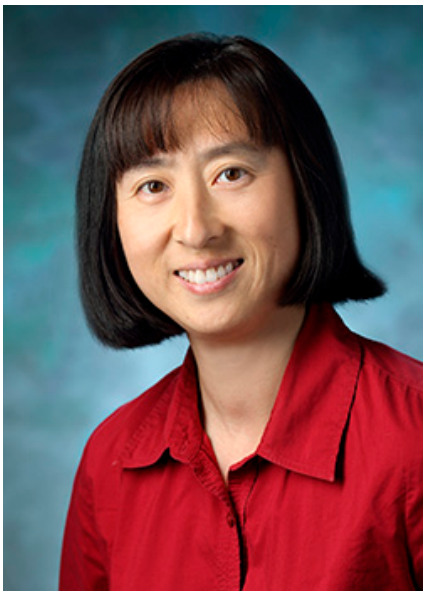

PHOTO COURTESY OF KEITH WELLER

Elizabeth Chen

When I started to look for postdoctoral positions, I was limited in my geographic choices because my husband had gotten a job offer in Texas. So I interviewed with a couple of groups in Texas, and I really liked Eric Olson's lab, which studies all kinds of muscles. At the time, much was known about the transcription factors that specify the muscle cell fate, but little was known about how committed muscle cells fuse to form multinucleate syncytia. The process of myoblast fusion in *Drosophila* is similar to skeletal muscle cell fusion in mammals, so I proposed to Eric that I was going to use *Drosophila* genetics in his lab, which is a mouse lab, to screen for mutants in *Drosophila* that are defective in myoblast fusion. He likes genetics, too, so he said, "Sounds great. Just go ahead and do it." [Laughs]

Is your husband also a scientist?

Yes. He studies the Hippo pathway. Later on we were lucky to both find positions at Hopkins, so things have worked out well for us. But with two careers and two kids, we definitely have a busy household, so we really treasure our family time together.

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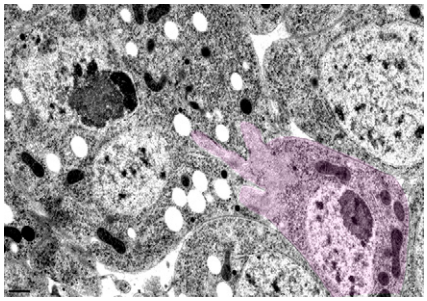

IMAGE COURTESY OF SHUIANG ZHANG

A fusion-competent myoblast (pink) presses finger-like protrusions into a founder cell.

What was known about muscle cell fusion when you began your postdoc?

A couple of cell adhesion molecules and a couple of intracellular proteins involved in the process had been identified in *Drosophila*, but there was no genetic or molecular pathway connecting these components. In mammals, tissue culture studies had implicated several classes of proteins in muscle cell fusion. Some of these proteins had been knocked out in the mouse, but in many cases—maybe due to redundancy or because these proteins weren't essential in vivo—the fusion process was unaffected. So it wasn't clear what was going on. It was pretty much a black box.

After two years working on a large-scale screen in *Drosophila*, I identified many fusion-defective mutants. *Antisocial* was the first gene that I picked to study because I had multiple alleles, and it turned out to function as an adapter protein that interacts with one of the cell adhesion molecules and with a regulator of the actin cytoskeleton. The second was *Loner*, which encodes a guanine nucleotide exchange factor for the small GTPase Arf. From these two studies we figured that fusion might have something to do with the actin cytoskeleton.

When I left Eric's lab to start my own lab at Hopkins, I still had more mutants to study. The first one that my lab cloned and characterized was a gene called *WASP-interacting protein* (*WIP*). It was *WIP* that led us to actin's role at the site of fusion.

PRESSING CLOSER

What did you see?

In myoblast fusion, a muscle founder cell attracts the surrounding fusion-competent myoblasts to attach to it, and then the founder cell and myoblasts fuse together. We found that WIP is only localized in the fusion-competent myoblasts and that it colocalizes nicely with a dense actin focus at the site of fusion. This suggested that the actin focus may be asymmetric. But at the time it was very hard to make that call definitively because the two cells' membranes are so close together at the site of fusion that we couldn't resolve them by confocal microscopy. We finally resolved the issue by expressing GFP-tagged actin in either the fusion-competent myoblast or the founder cell and then looking for colocalization with phalloidin. We found that only when GFP-actin was expressed in fusion-competent myoblasts did GFP-actin form a focus that precisely colocalized with phalloidin.

We were pretty sure at this point that the fusion interface was asymmetric, but we got a big surprise when we looked at the actin focus using electron microscopy. My postdoc called me from the EM facility and said, "Elizabeth, I saw finger-like protrusions on the fusion-competent myoblast!" From these EM studies, we showed that the myoblast forms a cluster of finger-like, actin-rich protrusions to invade the founder cell at the site of fusion. When this podosome-like invasive structure is defective, fusion pores don't form. Our hypothesis is that these dynamic, actin-propelled fingers serve to increase the contact area between the two cell membranes and push the two membranes into close proximity to promote cell fusion.

What are the minimum constituents of this process?

With that question in mind we wanted to reconstitute cell–cell fusion in a cell line that otherwise doesn't fuse. We started with *Drosophila* S2R+ cells because they have a very low basal level of fusion. Studies in *C. elegans* identified a fusogenic protein called EFF-1. We decided to see if EFF-1 could induce fusion in the S2R+ cells, but it only induced a low level of fusion. Next we tried coexpressing EFF-1 with a *Drosophila* cell adhesion molecule called Sns, and we saw a very high level of fusion. Using this system we determined that the actin polymerization machinery is required for Sns-enhanced fusion. We also observed podosome-like structures in the cell culture system that were similar to those we found in the *Drosophila* embryo. Therefore we think that this could be a general mechanism that other cell types use to mediate fusion.

We want to gain a deep mechanistic understanding of the fusion process. We would like to know what the fusogenic proteins are that mediate different types of cell–cell fusion events. We're also interested in looking at what happens at the other side of the fusion interface in the founder cell. How does it respond to the intrusion of the myoblast's podosome-like structures? Another direction of the lab is to look at myoblast fusion in higher animals, for example in vertebrate development and regeneration.

"We want to gain a deep mechanistic understanding of the fusion process."

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Chen's group at Johns Hopkins