Let curiosity lead you

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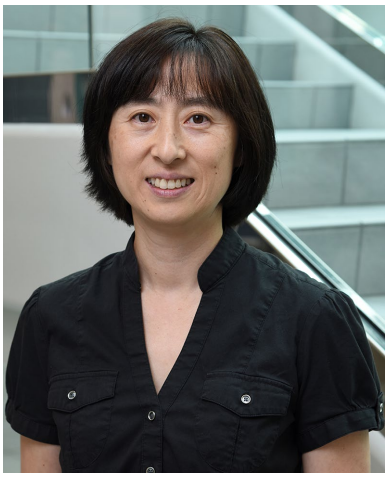
ABSTRACT **It is an incredible honor to receive the Woman in Cell Biology Mid-Career Award for Excellence in Research. My lab works on cell–cell fusion, an indispensable process in the conception, development, and physiology of multicellular organisms. In this essay, I reflect on my curiosity-led journey, which uncovered some unexpected mechanisms underlying cell–cell fusion.**

Science is an adventure. Facing the vast frontier of unknowns, how do we decide where to explore? In the history of science, many landmark discoveries were made simply because of a human being's inquisitive mind. In the process of trying to answer all of those whys, some of which may seem irrelevant to humans at the time, scientists have unveiled countless mysteries of nature and ourselves. It is these groundbreaking discoveries that provide the ingredients to translate into medically relevant treatments and therapies. Who would have imagined that studying the patterning of tiny bristles of the fruit fly larvae, the chromosomal ends of the freshwater pond critter *Tetrahymena*, and repeated sequences in bacterial adaptive immunity would lead to the discoveries of mechanisms underlying human development, stem cell maintenance, tumorigenesis, aging, and arguably the most powerful genome editing methods to date? Perhaps more relevantly, who can predict where the next most important discovery will emerge? One could say that we have now unearthed enough of the details that it is high time to translate our basic knowledge into practical cures. While I agree, I also believe that there will always be ample need for curiosity-driven research. There are so many whys that have not yet been answered, and only in their answering will we find the fresh ideas to later translate into cures for various diseases and to improve the well-being of mankind.



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My own curiosity about nature stemmed from my childhood in Jilin, a cold northeastern province of China. Every spring, I thrilled to see the first sprout of grass on the soccer field, the slow bloom of green across the thawing expanse. My favorite after-school pastime was climbing trees, and when I was high in the branches, I loved to observe the leaves and flowers up close. When I was a teen, I read an essay on Madame Curie in an English textbook at school, and was so charmed by her story that I read her biography on my own time. I was inspired by her relentless pursuit of discovery and her heroic labor of four years, the purification of radium in a shed. Her wise words still echo loud and clear: "…Neither do I believe that the spirit of adventure runs any risk of disappearing in our world. If I see anything vital around me, it is precisely that spirit of adventure, which seems indestructible and is akin to curiosity."

As I grew up, my curiosity shifted to human matters: how does an embryo become a body that can see, speak, smell, move, and think? What separates the human from the whale? How do we, in all our strength, fall so easily to disease? Fittingly, I ended up in the Biology Department of Peking University for my undergraduate studies. But before long, I found that I was not learning enough. In the late 1980s, China was just beginning to open its doors to the Western world, and science education was lagging behind by decades. Our cell biology textbooks were two 6 × 8 pamphlets with just over 100 pages each. We were taught to memorize facts instead of learning how the facts were discovered. So I decided to study biology in the United States. After taking graduate classes in many different areas at UCLA, I fell in love with the beautiful gene

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expression patterns of *Drosophila* embryonic development. This led me to the Developmental Biology Department at Stanford University to further pursue my interest.

For my thesis work, I joined the lab of a great *Drosophila* geneticist, the late Bruce Baker. Bruce had a long-standing curiosity about sex determination in *Drosophila* and elucidated a novel regulatory pathway involving RNA splicing, the first of its kind. Through his example, I learned that the road less traveled may give the greater reward, and that questions should be answered by any possible means—even if the method lies beyond one's field, as molecular biology did for Bruce, a die-hard geneticist. In the spirit of exploration, I decided to start a new direction in the lab, exploring how the sex determination pathway is integrated with the general patterning genes to generate the sexually dimorphic tissue structures. As a young and naïve second-year graduate student, I was intimidated by the prospect of initiating a new project, but the excitement of exploring uncharted territory snuffed out my fears and kept me going on this less traveled road.

For my postdoc studies, I headed again into the unknown. When I was looking for an interesting but poorly studied problem, cell–cell fusion caught my attention. Our lives begin with the fusion of sperm and egg, and our skeletal muscle fibers are multinucleated due to the fusion of myoblasts, yet at the time very little was known about the process of cell–cell fusion. I asked Eric Olson, my postdoctoral mentor, whether I could study myoblast fusion using *Drosophila* as a model in his lab. He encouraged me to work on it. It would take two years to conduct a large-scale forward genetic screen, a long and laborious process that led me to stay in the lab on Christmas Day, setting up hundreds of fly crosses. And the work paid off. Since such an unbiased systematic screen for myoblast fusion mutants had never been done before, I was able to isolate a dozen new fusion-promoting genes that would lay the foundation for my career.

Science always takes us to unexpected places. While I was expecting to find SNARE-like transmembrane proteins that mediate cell membrane fusion, the first two genes I characterized in the Olson lab hinted at a potential involvement of the actin cytoskeleton (Chen and Olson, 2001; Chen *et al.*, 2003). Subsequently, the first gene characterized in my own lab at Johns Hopkins University unambiguously demonstrated the requirement for the actin cytoskeleton in promoting fusion (Kim *et al.*, 2007). This was a surprise, since no one at the time expected the intracellular actin cytoskeleton to have any specific function in promoting cell membrane fusion. Probing this further, we observed a dense actin structure at the site of fusion between two myoblasts, and intriguingly, this structure appeared to be predominantly localized to one of the two fusion partners (Kim *et al.*, 2007). For a while we were perplexed by this potential asymmetry and continued looking for ways to clarify it. Eventually, using genetic tricks and electron microscopy, we demonstrated that each actin structure is made of an actin core and multiple figure-like protrusions generated exclusively in one of the two fusing partners (attacking cell) (Sens *et al.*, 2010). These protrusions project deeply into the other fusion partner (receiving cell) to facilitate cell membrane juxtaposition and fusion. Guided by this enlightening finding, we were able to reconstitute cell–cell fusion in cultured cells that normally do not fuse, by introducing two exogenous components into the cells: a cell adhesion molecule that triggers the formation of invasive protrusions and a transmembrane fusogen that we borrowed from the roundworm *Caenorhabditis elegans*. We found that cell fusion in this culture system is also mediated by finger-like protrusions, and that the function of these protrusions is to push the membranes into close proximity to engage the fusogen (Shilagardi *et al.*, 2013). It is satisfying to see that similar invasive protrusions were later found to mediate the fusion of mammalian myoblasts and osteoblasts (Shin *et al.*, 2014). Thus, using *Drosophila* embryos as an experimental model, we have uncovered a conserved and general mechanism underlying cell–cell fusion.

Ever since we discovered the asymmetric invasive structure at the site of fusion, which we refer to as the fusogenic synapse, we had been curious as to how the receiving cell responds to the invasive forces. Our genetic analyses again led the way. We found that the actin motor myosin II and the membrane skeleton protein spectrin are required for myoblast fusion (Kim *et al.*, 2015; Duan *et al.*, 2018). Strikingly, both myosin II and spectrin are specifically enriched at the fusogenic synapse on the side of the receiving cell. Teaming up with biophysicist colleagues Doug Robinson and Dan Fletcher, we found that both myosin II and spectrin exhibit mechanosensitive accumulation in the receiving cell in response to the invasive protrusions from the attacking cell. The locally accumulated myosin II and spectrin, in turn, increase the mechanical tension at the fusogenic synapse to further facilitate cell membrane juxtaposition and fusion. Suffice it to say, we would not have gotten to where we are today had we not extended our analyses from genetics to cell biology and to biophysics, which highlights the importance of using any possible means to answer questions, a lesson that I learned in graduate school.

After 20 years of exploration, cell–cell fusion is no longer the black box it used to be. It has been fun and exciting for us to uncover the intricate interactions between fusion partners by following our curiosity and solving problems with a multidisciplinary toolbox. Does our curiosity-driven research have something to do with understanding and treatment of human diseases? The answer is likely yes. Indeed, one of the fusion-promoting genes that we are studying right now has been implicated in a type of congenital myopathy characterized by poorly fused muscle fibers. I believe that our basic mechanistic studies will provide fresh ingredients to translate into clinical applications in the future. For now, we will continue to enjoy the amazing views along the less traveled path and let curiosity lead us on.

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