ACS chemical biology

Illuminating Cellular Signaling

The intracellular environment is an incredibly dynamic place, with new signaling molecules being born, dying, interacting, and transforming every minute. However, the roles and actions of many of a cell's signaling molecules remain a mystery. If researchers could light up the dark and shrouded inner environment of a cell, then they could observe cellular signaling in real time. This goal is the focus of work by Jin Zhang of the Johns Hopkins University School of Medicine in Baltimore and her colleagues. Zhang's team has created several fluorescent sensors that give a visual element to the action of intracellular signaling molecules; as they move inside cells, the sensors allow researchers to track and monitor particular signaling events. By shining this spotlight on cellular signaling molecules, Zhang's work is giving unprecedented insight into signal transduction events inside living cells.

In the Family. Zhang was born in 1972 in Beijing, China, to parents who were both engineering professors at Tsinghua University. Her parents encouraged her and her older sister, Qing Zhang, to follow their own interests, including writing stories and reading from the family's personal minilibrary of Chinese literature classics and science fiction books.

However, notes Zhang, "Science and engineering run in the family, and the family influence was pretty strong." She recalls many dinner discussions during her childhood that focused on why natural phenomena occur. If her parents couldn't answer her or her sister's questions, they encouraged the children to find solutions in books, through logical thinking, or by experimentation. "It became a fun thing to ask why and look for answers. That got me interested in science as a career," says Zhang.

As she progressed through the Chinese school system, Zhang excelled in her science classes and had particular interest in life sciences. By the time she was ready to enter Tsinghua University, she was certain that she'd choose biology as a major. However, her parents encouraged her to seek out advice before making a decision with such long-term consequences. Chinese students must settle on majors before entering a university, a choice that can be difficult to amend once they're enrolled. Zhang received some life-changing counsel from a friend her father made in the late 1970s when he was a visiting scholar at the University of California, Berkeley. "He told me that to go far in understanding biological systems at a molecular level, one needs to build a chemistry foundation," she says. With that in mind, she enrolled in the university as a chemistry student the following fall.

At the time, Tsinghua University offered only a physical chemistry major, which didn't offer Zhang many chances to indulge her love of the life sciences. She took opportunities where she could find them, however, such as joining a research project in which she used analytical approaches to study various Chinese herbs. Zhang notes that starting her academic training in physical chemistry gave her a strong background in quantitative analysis, a theme that continues to influence her work today.

As she neared the end of college, Zhang began to research graduate programs, focusing on those in the U.S. "After 23 years on the same campus, practically growing up at Tsinghua University, I was ready to go away for graduate school," she says. Zhang adds that she was also ready to reintroduce biology back into her studies. Looking for programs that focused on the interface be-

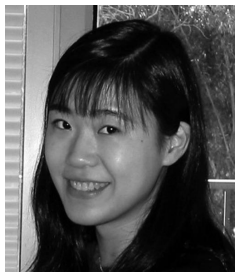

Image courtesy of Jin Zhang.

Profiles provide insights into the lives, backgrounds, career paths, and futures of scientists who serve as Experts on *ACS Chemical Biology*'s online Ask the Expert feature. Dr. Zhang will begin answering your questions in mid-April, 2007. Readers are encouraged to submit questions to the Experts at www.acschemicalbiology.org. The editors will post the most interesting exchanges on the web site.

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tween chemistry and biology, she found her match at the University of Chicago in the laboratory of David Lynn.

The program didn't offer the option of lab rotations. After Zhang was accepted at the university, she and other new students met with professors and then chose their advisers on the basis of this interaction. Zhang remembers that she immediately got a positive feeling from Lynn and the students in his lab, who seemed well supported, encouraged, and excited about their work.

After Zhang joined Lynn's team, he immediately asked her to work on a project to investigate the mechanism behind signal-induced pathogenesis by *Agrobacterium*, a species of bacteria that attacks some types of plants. The bacterium was well studied because it is the only known natural vehicle for incorporating foreign genes into higher plants. However, researchers didn't understand why some plants, such as corn, are able to resist *Agrobacterium* transformation.

For her thesis work, Zhang and her colleagues discovered a chemically labile molecule secreted by corn plants that impedes *Agrobacterium* virulence (*1*). The researchers isolated the molecule, made analogues of it, and then used these analogues to investigate how virulence was initiated through the bacterial two-component system and how this plant molecule was able to inhibit the virulence process (*2*). The question of how far the molecule can spread away from the plant and inhibit the bacterium prompted Zhang to build a mathematical model to describe the molecule's diffusion gradient. As a result of a collaboration on the model between Zhang and her father, whose expertise in mathematical modeling has helped the engineering of many of China's large dams, her father ended up being a coauthor on the resulting publication (*1*). "It was kind of cool to publish with my dad," says Zhang.

Seeing is Believing. Toward the end of her doctoral training, Zhang was interested in exploring signaling mechanisms in more complex organisms, such as those in mammalian systems. She began auditing biology courses to build up her life sciences knowledge, and she became a regular attendee in seminars given by both the chemistry and biology departments.

Zhang was especially inspired by one particular seminar, given by Roger Tsien of the University of California, San Diego (UCSD). Tsien's work centered on engineering molecular tools to dissect signaling events in cells. That day, he spoke about various reporters that he and his colleagues had crafted, including a fluorescent sensor that provides a readout of intracellular calcium dynamics. Another molecule Tsien's team designed fluoresces after binding to tetracysteine motifs inside cells, giving researchers a genetically targetable probe to follow proteins throughout cells.

Zhang was captivated by the idea of being able to visually monitor molecular events inside living cells. Thinking ahead to her postgraduate training, she contacted Tsien to inquire whether he had any open postdoctoral positions. Despite her relatively unrelated background, Tsien seemed impressed by Zhang's intense interest in his team's work, and he invited her to join his lab.

Zhang's first project as part of Tsien's group involved developing a strategy for visualizing kinase activity with high spatial and temporal resolution in living cells. "Fortunately, a leading expert on kinases worked just downstairs from Tsien's lab," says Zhang. The expert, Susan Taylor, agreed to become Zhang's coadviser after Zhang approached her to collaborate on the project.

Using molecular biology techniques, Zhang designed a variety of FRET-based sensors and then tested each construct in live HeLa cell cultures. "I went through about 20 different constructs, but I didn't see anything. One day, these one or two cells started changing color before my eyes," she recalls. The color change signaled that a phosphorylation event had occurred inside the cell—a sure sign that her construct worked (*3*).

Zhang credits her quick success, which took place only a few months after she joined Tsien's lab, to a host of productive collaborations, including work with Tsien's UCSD colleague Steve Adams and with then-postdoctoral-fellows Alice Ting and Robert Campbell. Once other researchers learned about the new kinase-sensing construct, Zhang formed other collaborative relationships to extend the general strategy to other kinases and to broaden her tool's applications. One of her first partnerships was with UCSD medical faculty member Jerry Olefsky and his then-postdoctoral-fellow Chris Hupfeld.

Olefsky and Hupfeld had recently discovered that chronic insulin administration prompts cells to overproduce cAMP. After creating an improved protein kinase A (PKA) reporter, Zhang and her new colleagues found that chronic insulin treatment delays PKA-mediated phosphorylation (*4*), a highly unexpected finding in light of Olefsky and Hupfeld's previous work. "Sometimes, surprises can lead to even more discoveries," says Zhang. The team later discovered a specific coupling between PKA and a receptor that activates cAMP-producing machinery.

Business of Discovery. After three years as a postdoctoral fellow, Zhang was ready to search for a faculty position. She notes that her goal was to build a lab with the same qualities that she valued during her years of graduate and postgraduate training. "Those two labs were very collaborative and interactive," she says. In the fall of 2003, Zhang opened her laboratory at Johns Hopkins School of Medicine in Baltimore. "Hopkins was a really good fit for building the lab that I had in mind," she adds.

With appointments in the departments of Pharmacology and Molecular Sciences, Neuroscience and Oncology, she finds herself surrounded by colleagues who are inter-

ested in elucidating the mechanisms of signaling events and understanding the misregulation that can lead to disease—both biologists and chemists. "It's the best of both worlds," says Zhang.

Over the past four years, she and her collaborators have continued the theme that Zhang developed during her postdoctoral training: creating innovative molecular tools to study signal transduction in living systems. Her lab's first independent publication involved developing a sensor to visualize and quantify cAMP dynamics (*5*).

"We had this idea that we could sandwich a cAMP binding protein between a FRET pair, cyanic and yellow fluorescent protein," says Zhang. She notes that one of her first graduate students, Lisa DiPilato, was instrumental in getting the project to work. DiPilato generated a successful construct and tested it in living cells. With Zhang's guidance, she and her colleagues provided the first direct measurement of rapid cAMP accumulation in mitochondria in living cells.

In another recent project, Zhang, her postdoctoral fellow Bharath Ananthanarayanan, and research associate Qiang Ni designed a sensor that used a pseudoligand to sense phosphoinositide. The team engineered a short peptide made of acidic residues to bind to the same domain as the real phosphoinositide ligand. When the real ligand competed the pseudoligand away, the protein's conformational change generated a FRET signal (*6*). "We can really follow the spatial and temporal dynamics of this event," says Zhang.

She notes that her team is working to create additional reporters to follow signaling events both downstream and upstream of those they're already able to visualize. Eventually, this approach could yield a systems-biology-style map with space and time information for various types of signal transduction events. Once she and her colleagues have gathered sufficient information about what goes on in a healthy cell to achieve a quantitative understanding of cellular signaling networks, Zhang adds that she would like to compare this normal signaling activity profile with cellular models of diseases such as cancer, diabetes, and obesity. With the addition of high-throughput techniques developed recently (*7*), such work could someday lead to the discovery of drugs to treat these conditions.

With her many ideas for upcoming projects, Zhang notes that she's looking forward to an exceptionally busy and exciting future. She recalls that when she first started her faculty position, a colleague presented her with a gift: a script of Chinese writing with an English translation.

"The English version reads, 'Discovery is our business,'" says Zhang. "The best part of this business is that it will never be done. Discovery never has an end."

— Christen Brownlee, Science Writer

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