ACS chemical biology

Sheng Ding: Using Chemical Methods To Control Cell Fate

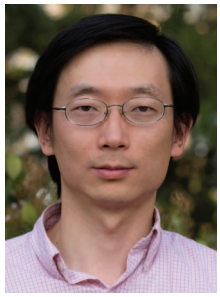
For most of the study of developmental biology, differentiation has been thought to proceed directly down a one-way street: cells progress from the totipotent zygote to pluripotent stem cells to more specialized cells with dedicated functions, without the option of backtracking. Lured by the promise of regenerative medicine and the fundamental challenge of better understanding this age-old pathway, developmental biologists have focused their concentration on finding ways to control cell fate, such as turning back the clock through genetic means or harnessing endogenous mechanisms to steer cells toward desired functions. However, these efforts have highlighted how truly unwieldy controlling cell fate can be. Seeking new and better ways (*1*) to guide this biological phenomenon, Sheng Ding, Ph.D., took advantage of his training as a chemist. At his laboratory at The Scripps Research Institute in La Jolla, CA. Ding and his colleagues use combinatorial chemistry to develop large libraries of small molecules and screen them for their ability to influence cell fate in a variety of ways, such as holding stem cells in a state of self-renewal (*2, 3*), dedifferentiating somatic cells to an earlier developmental state (*4–7*), or precisely directing differentiation of stem cells to desired lineages (*8–10*). By using chemical tools, Ding and his colleagues are succeeding in influencing the course of stem cell biology.

Budding Chemist, Turned Biologist. Ding was born in 1975 in Beijing, China, to a mother who is a physician and a father who is a high-energy physicist at Peking University. He suspects that growing up in such a scientifically and academically oriented family played a heavy part in influencing his own career, as well as that of his brother, who now works as a chemist for Novartis. Ding remembers his whole family being interested in the great outdoors and spending plenty of time enjoying nature. He also remembers being fascinated by chemistry as early as elementary school, when teachers showcased the field's magic through simple color-change reactions.

Chemistry continued to intrigue Ding through high school, where he enthusiastically pursued his interest by participating in the International Chemistry Olympiad, an annual academic competition for high school students interested in chemistry around the world. He also began studying college-level chemistry on his own, eager to independently learn as much as he could.

By the time Ding finished high school, he was sure that he'd continue studying chemistry in college. He was also certain that he'd probably continue his studies in the United States, the country widely recognized as one of the best places worldwide for higher education. Ding applied to several American schools, but a generous scholarship offer from the California Institute of Technology (Caltech) quickly narrowed his choices. After accepting Caltech's offer, he headed to Pasadena the fall of 1996.

Ding remembers early college as an exciting time full of opportunity, with no trepidation about moving far from home to a highly competitive school in a foreign country. However, when his classes began in earnest, he realized how tough college would be. Ding says that he was soon glad for the independent study skills he'd picked up in high school. "When I came to the United



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States, my English wasn't very good. I have to say that at the time, I couldn't really speak much or understand much in the classroom, so I spent hours reading textbooks," he recalls. "Just like in high school, I taught myself a lot of things."

As his knowledge of chemistry progressed, Ding felt like studying biology was "a natural progression". He began mixing a few biology classes into his chemistry-heavy lineup. He also became interested in studying with chemistry professors who blended biology into their own work. At Caltech, a school known for its outstanding undergraduate research programs, Ding and his classmates were encouraged to assist seasoned researchers with their own projects during summers and hours outside of class, giving students a taste of producing original research and publishing papers. In Ding's first experience with undergrad research, he worked with Sunney Chan, Ph.D., a now-retired biophysical chemist who used a variety of physical and chemical methods, including various spectroscopy, chemical modification, and site-directed mutagenesis, to investigate the structure and function of proteins. "It was one of my earliest steps into biology, my chance to start really thinking deeper into biological questions," says Ding.

In his next research experience at Caltech, Ding was able to continue delving into biology while getting some rigorous chemistry training. In the laboratory of Andrew Myers, a synthetic organic chemist who is now a professor at Harvard University, Ding assisted in total syntheses of complex natural products important in biology and human medicine. The painstaking work of organic synthesis was good preparation for learning the patience and stamina necessary for a research career, Ding says. "Total synthesis is a long race in a way, scientifically and mentally. I felt like I really matured during that time," he recalls.

After Myers was recruited to Harvard, necessitating the search for another research laboratory, Ding found an opportunity his senior year with Robert Grubbs, an organometallic chemist whose work on olefin metathesis catalysis eventually netted him the 2005 Nobel Prize in Chemistry. Ding was able to expand his chemistry knowledge in new ways there, working on developing the second generation of Grubbs' catalyst for olefin metathesis (*11*). In his last year at Caltech, carrying a light course-load, Ding was able to pile on even more research experience in another two laboratories concurrently. He worked both for Douglas Rees (*12*), an expert in using X-ray crystallography to study protein structure and function, and William Goddard, who uses computational methods to study atomic and molecular interactions, including interactions between small molecules and biological macromolecules.

"By the time I finished college, I was involved more and more with biology," remembers Ding. "I was exposed more and more to biological ideas, the biology literature, and real-world biology questions."

However, he adds, the vast majority of his biology knowledge was gleaned informally, learned as he participated in research rather than strictly from biology classes. To this day, he asserts, such informal training allows him to approach biological questions in different ways than his colleagues with more typical training in the life sciences. "My lack of formal biology training was good and bad—it took me quite awhile to think as a biologist. Because I'm a chemist, I come at questions from a chemical aspect," says Ding. "Now I think it's one of my biggest advantages. I think differently about things."

Asking Naïve Questions. By the time Ding was ready to apply to graduate school, he was determined to focus his future research on chemical biology. He applied to several competitive institutions, but the work of Peter Schultz, Ph.D., made his laboratory at The Scripps Research Institute Ding's top choice. Ding says he was drawn to Schultz's widely diverging interests, such as generating and characterizing catalytic antibodies, engineering proteins with unnatural amino acids and using chemical libraries to probe basic biological questions and generate promising leads for pharmaceuticals.

Ding contacted Schultz by email to relay his interest, which ended up being mutual; Schultz, who had also received his undergraduate and Ph.D. degree from Caltech, sent the aspiring prospective graduate student a large packet of his publications within a week of receiving Ding's email, along with a letter expressing his enthusiasm about Ding joining the laboratory. Ding recalls that his singular interest in Schultz almost cost him admission to Scripps. "In retrospect, I perhaps didn't show equal interest when I was meeting other researchers," he says. "I was already so determined to do research with Schultz."

In the end, however, he was accepted at Scripps and began working in Schultz's laboratory in the summer of 1999. While he was mulling over what research projects to begin in this new setting, Ding says that Schultz invited him to participate in a thought experiment. "We have these functional genomics tools—microarrays, tools for high-throughput screening—right at our hands. What would be the most fascinating things we can do with these new technologies?"

After giving Schultz's question some consideration, Ding had an answer: he had become fascinated by new research in biological development, especially stem cell research. With human embryonic stem cells isolated just a year earlier, in 1998 in James Thomson's laboratory at the University of Wisconsin, the field of stem cell biology seemed ripe for discovery. Though Schultz's laboratory did not yet have a project that focused on stem cells, Ding reasoned that it could be the perfect setting for making new discoveries in novel ways.

At the time, what little was known about stem cells still dictated that these undedicated cells proceeded on a straightforward path to a more differentiated state. However, Ding says he and Schultz had "crazy ideas about impossible things. We asked incredibly naïve questions." One of the questions he and his mentor eventually fixed upon was whether they might influence cells to reverse in development and proceed backward toward a more undifferentiated state.

"People thought this was impossible—a very fundamental concept of biology is that cells cannot go backward in development," says Ding. "But we weren't influenced by authority or textbooks. We thought, why can't we make this happen with chemicals? Why not?"

Ding and Schultz's wonderings were not completely naïve. Ding notes that evidence in some lower organisms, such as amphibians, indicate that the regeneration process these organisms take advantage of to regrow lost limbs or tails depends on cells backtracking in development, then redifferentiating into the desired structures. However, this phenomenon did not seem possible in mammals.

Ding and Schultz decided to develop chemical screens to find out if small molecules might induce dedifferentiation after all. Using combinatorial methods, the researchers developed large chemical libraries of over 200,000 small molecules, then used high throughput screening assays to test each chemical's effects on differentiation in cell-based assays. The painstaking work eventually netted the researchers a huge prize: one of the small molecules, which the researchers named "reversine," caused a myogenic-committed cell line to reverse track in development, dedifferentiating into multipotent progenitor cells that could be redifferentiated into osteoblasts and adipocytes (*4*). This work, published soon after Ding completed his Ph.D. in 2003, was a revolutionary finding for stem cell biology. For the first time, researchers had taken advantage of high-throughput screening to identify small molecules that influenced cell developmental fate.

By the time Ding had finished his degree, he and Schultz had amassed even more promising leads for small molecules that had the potential to affect cell fate. With help from Schultz, Ding was offered an assistant professorship position to further expand research areas in stem cell biology using chemical approaches. "It was a really wonderful opportunity, so rare and so great," remembers Ding. "I was really lucky. I thought, I really can't not take this opportunity."

While his own new laboratory space was being renovated in the fall of 2003, Ding recalls that he had little downtime. He quickly initiated a couple of new discovery projects in collaboration with Schultz, including a small molecule he and Schultz later dubbed "neuropathiazol." Two years after he began his new position, Ding, Schultz, and their colleagues published research showing that this novel small molecule potently and selectively induced neural progenitor cells to differentiate into neurons (*8*). The work was one of the first showing that synthetic small molecules, rather than endogenous growth factors and other proteins, could precisely direct adult stem cell differentiation.

Making the Impossible Happen. Once Ding's independent laboratory was up and running, he began recruiting his own students and postdoctoral fellows and developing new projects that exploited small molecules to answer developmental biology questions in new ways. One of the most pressing first questions for Ding and his colleagues was how to maintain embryonic stem cells in an undifferentiated state in a chemically defined environment. Though current protocols suggested that the feeder cells used to coat stem cell culture dishes, as well as a mixture of serum and specific growth factors, were sufficient for maintaining stem cells, the largely uncharacterized slew of molecules present in stem cell media often led to enormous variability in stem cell potential from batch to batch.

Seeking a solution, Ding and his collaborators searched for a small molecule among their chemical libraries that might be sufficient for suspending mouse embryonic stem cells in an undifferentiated state. In 2006, the researchers published evidence that a promising molecule they named "pluripotin" acted much like feeder cells and complex serum, preventing the stem cells from progressing in development (*2*). Further investigation showed that the molecule appears to block the activity of two proteins known to be involved in differentiation, RasGAP and ERK1.

In another project, Ding's laboratory has worked on improving the methods that other research teams had developed to dedifferentiate fibroblasts back into pluripotent stem cells. Previously, a Japanese team had accomplished the same feat by using viral methods to insert four genes, two of which are known oncogenes, into the fibroblasts' genomes. Since methods that use oncogenes and imprecise genetic manipulation will not be suitable for therapeutic purposes, Ding and his colleagues looked for small molecules that might be able to replace those genes. In 2008, the researchers published early work suggesting that they could recreate the Japanese researchers' success using just two of the inserted genes and a cocktail of two small molecules (*6, 7*). Their goal, says Ding, is to eventually replace the need for these genes entirely, completely dedifferentiating fibroblasts using a cocktail of small molecules. His laboratory is well on the way toward this goal.

Also last year, Ding and his colleagues had success using another combination of genetic reprogramming and treatment with small molecules to generate novel rat and human pluripotent stem cells, overcoming a problem more than a decade old (*13*). Their new technique could eventually help researchers develop cells to create lines of

a variety of transgenic animals as thoroughly as they've created transgenic mice, says Ding.

Despite his slew of early successes, Ding points out that the field of stem cell biology and regenerative medicine is still very young. "We still lack very basic understanding of stem cell mechanisms—we lack very precise control over cell fate," he says. Consequently, he and his colleagues plan to continue asking and answering questions to explore this field for the long-term, using small molecule chemistry as their primary tool. Eventually, Ding hopes to develop some of the molecules that he and his colleagues have developed into therapeutics that might target the body's own sources of stem cells for regeneration. He and his colleagues have started their own biotech company, Fate Therapeutics Inc., to usher promising molecules through the pharmaceutical pipeline.

In the distant future, Ding says, pharmaceuticals may be able to help humans achieve the same regenerative heights that lizards have long maintained. In the meantime, though, his personal rewards come from satisfying his curiosity and continuing to defy conventional wisdom.

"By continuing to ask naïve questions and putting in hard work, we are making impossible things happen," he says.

—Christen Brownlee, Science Writer

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