

A Key in Search of a Lock

Prodding stem cells to be neurons.

One of Sheng Ding's favorite activities outside the lab is scrambling up the twisted granite boulders of Southern California's Joshua Tree National Park. By finding just the right combinations of grips, foot placements, and body English, Ding can mold his body to the cracks and outcroppings, surmounting virtually any obstacle he encounters. It's a similar power of combinations that excites him about chemistry.

Earlier this year, Ding, then an HHMI predoctoral fellow at The Scripps Research Institute, combined a set of chemical building blocks to assemble a molecule that can spur embryonic stem cells to become neurons. Now an assistant professor of chemistry at

toward specific cell fates must pass through carefully secured gateways. And the keys to those doors are the signaling molecules that traverse between and within stem cells, instructing them to differentiate into muscle, intestine, bone, or any other cell type. In an attempt to unlock the gateway toward a nerve-cell fate, Ding and his colleagues designed more than 50,000 small-molecule "keys" and tried them all, one by one.

Starting with 10 different synthetic "core scaffold" molecules, the researchers added various combinations of atoms and chemical groups to create a wider and more diverse set of compounds. They divided those molecules and then attached still

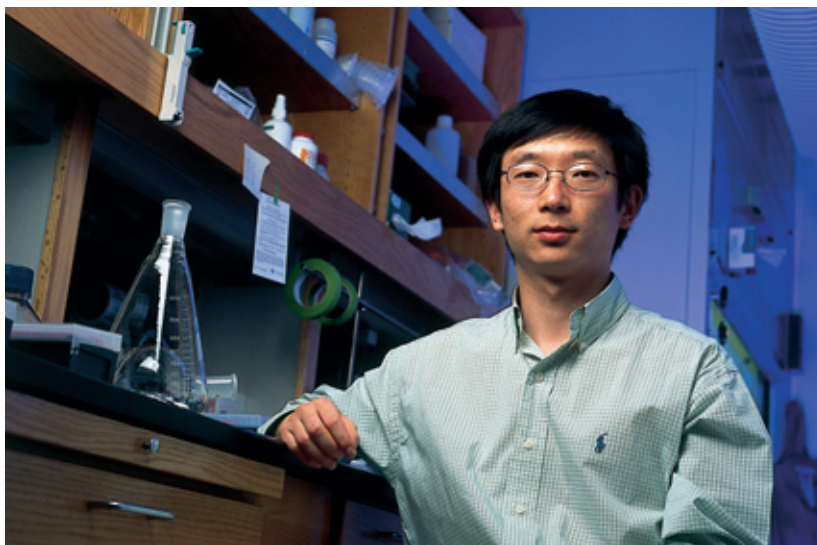
new compounds by further tweaking the structure of the best candidate. One of those fine-tuned compounds, dubbed TWS119, proved particularly potent for stimulating mouse embryonic stem cells to become neurons. Cells bathed in the chemical developed the characteristic wiry network of axons and dendrites that neurons use to transmit their electric impulses. The cells also tested positive for proteins and other molecules found only in neurons or cells on their way to becoming neurons.

How did TWS119 perform its magic? The molecule's shape and chemical properties enabled it to bind to a molecule that controls stem cell differentiation, and that binding cleared the path to the neuronal cell fate. Ding and his colleagues had identified a key in search of a lock, exploiting the binding power of TWS119 to find the molecule with which it interacts—that is, the "lock" itself. In a technique called affinity chromatography, they attached molecules of TWS119 to tiny beads and then mixed the beads with stem cell extracts. Whatever molecules in the extract that could bind to TWS119 should then have become tethered to the beads. After the researchers gently washed the beads to remove all nonbound molecules, one prominent protein stayed attached.

That protein, called glycogen synthase kinase-3 β (GSK-3 β), turned out to be a familiar one to biologists. Protein kinases, enzymes that attach phosphate groups to other proteins, are a major class of cellular signaling molecules. Biologists had recognized GSK-3 β 's role in many other aspects of cellular regulation, but its involvement in nerve cell differentiation had only been hinted at. TWS119 inhibits GSK-3 β 's enzymatic activity, but Ding says that more work is needed to figure out exactly why this leads stem cells to become nerve cells. "TWS119 is just our first step to demonstrate that we can rationally screen small molecules to identify interesting leads," he explains.

Developing TWS119 is only the latest of Sheng Ding's many accomplishments since emigrating from China in 1996. He graduated from the California Institute of Technology in 1999 with a host of honors and is author or coauthor of 15 scientific papers, with several more on the way.

—PAUL MUHLRAD



ROBERT BURROUGHS

Sheng Ding uses the power of chemical combinations in research he hopes will lead to treatments for regenerating damaged or diseased organs.

Scripps after just having completed his Ph.D., the 28-year-old chemist hopes his research will eventually lead to treatments for regenerating patients' damaged or diseased organs. Ding and his colleagues at Scripps and the Genomics Institute of the Novartis Research Foundation reported their findings in the June 24, 2003, issue of the *Proceedings of the National Academy of Sciences (PNAS)*.

Embryonic stem cells are open to pursuing almost any destiny, but the paths

other side groups, repeating the cycle once more until they had created thousands of distinctly shaped chemicals, each separated in its own bar-coded reaction vessel.

Using a robotic workstation, team members added a sample of each compound to mouse embryonic stem cells growing in 384-well culture plates. Initial tests identified several promising molecules that seemed to initiate the process of turning the cells into neurons. Ding and colleagues then made 50