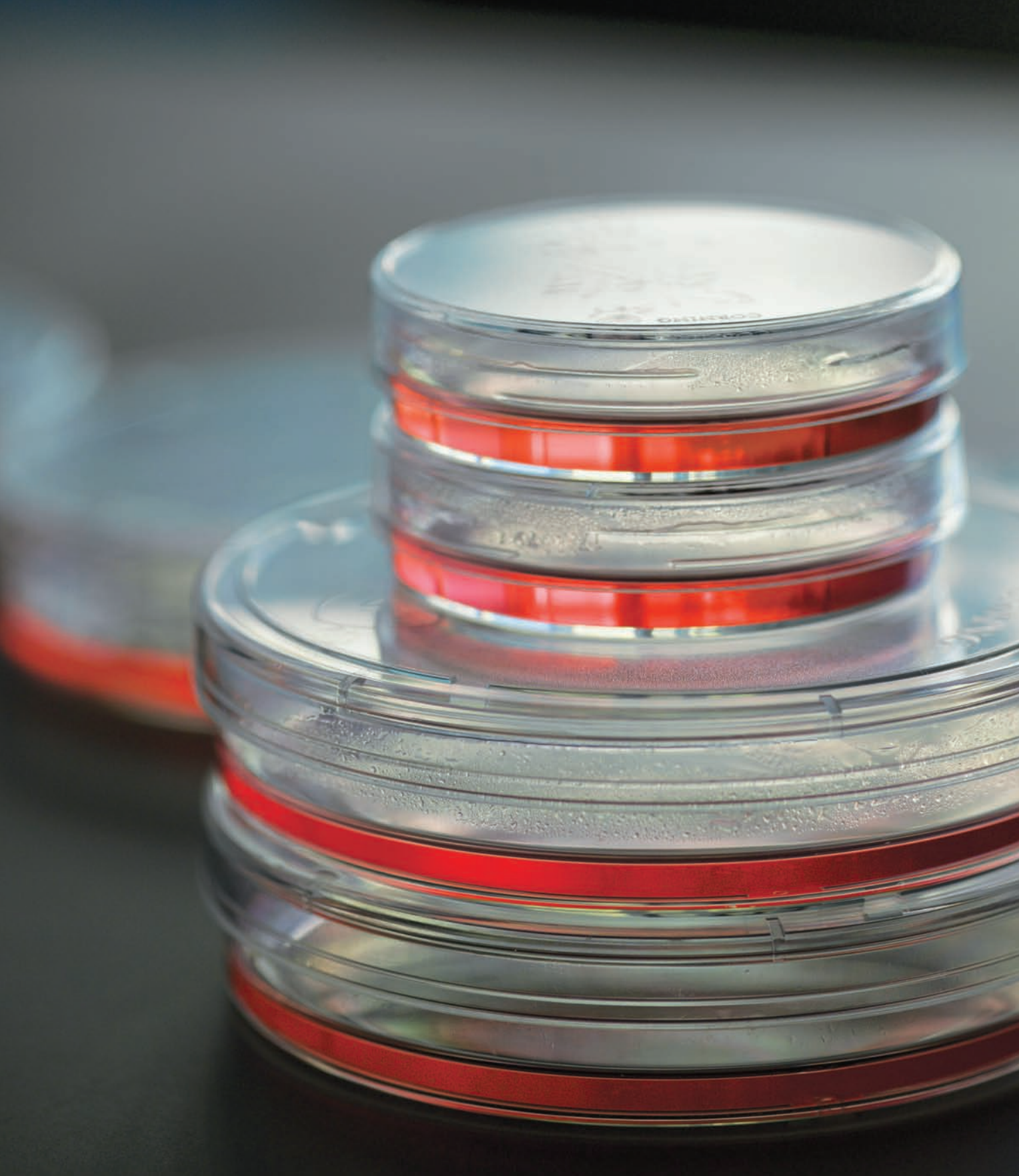


After stem cells grow for 30 days in culture medium (*red*), they become specialized tissue that can be used to model different diseases.

MEDICINE

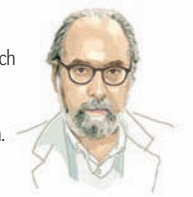
diseases in a dish

A creative use of stem cells made from adult tissues may hasten drug development for debilitating diseases

By Stephen S. Hall

Photographs by Grant Delin

Stephen S. Hall described the early history of stem cell research in the award-winning *Merchants of Immortality* (Houghton Mifflin, 2003). His most recent book, *Wisdom: From Philosophy to Neuroscience* (Vintage), will be issued in paperback in March.



ON JUNE 26, 2007, WENDY CHUNG, director of clinical genetics at Columbia University, drove to the New York City borough of Queens with a delicate request for the Croatian matriarchs of a star-crossed family. She asked the two sisters, one 82 and the other 89, if they would donate some of their skin cells for an ambitious, highly uncertain experiment that, if it succeeded, promised a double payoff. One, it might accelerate the search for treatments for the incurable disease that ran in their family. Two, it might establish a valuable new use for stem cells: unspecialized cells able to give rise to many different kinds of cells in the body. "We had a very nice lunch and literally went back to the house and took the biopsies," Chung remembers. As they sat around the dining-room table, the elderly sisters were "very happy sticking out their arms," recalls the daughter of the 82-year-old woman. The younger sister told Chung: "I get it. Go right ahead."

The sisters suffered from amyotrophic lateral sclerosis (ALS), a degenerative and slowly paralyzing nerve disorder that is also known as Lou Gehrig's disease, after the Yankee slugger who was told he had it in 1939 and died two years later. The 89-year-old showed few signs of the disease, whereas her 82-year-old sister had trouble walking and swallowing.

Although most cases of ALS are not hereditary, the disorder has struck multiple members of this particular family. Affected members inherited a mutation that has been linked to a more slowly progressing form of the disease than the one that attacks most other people with the condition. Chung had been tracking the disorder across several generations of the family in Europe and the U.S. "Lou Gehrig's disease is not a pretty way to die," she says. "Every time family members would get together at funerals, people in the younger generation would be looking around and asking, 'Am I going to be next?'"

It took Chung just a couple minutes to perform the actual "punch biopsy"—two quick nips of flesh, each three millimeters in diameter, from the inner arm. Eventually the sisters' cells, along with skin samples from dozens of other ALS patients and healthy volunteers who similarly donated bits of tissue, were chemically induced to become a form of stem cell known as an induced pluripotent stem cell and were then reprogrammed to become nerve cells. Specifically, they were induced to become motor neurons, the nerve cells that directly or indirectly control the muscles of the body and are adversely affected by ALS. The resulting tissue cultures exhibited the same molecular defects that gave rise to ALS in their human donors. In other words, the investigators had, to an astonishing extent, re-created the disease in a petri dish.

With these cells in hand, they could begin to study exactly what goes wrong in the nerve cells of ALS patients and could start to screen potential drugs for useful effects on the diseased cells. This use of stem cells is new and contrasts with so far disappointingly slow progress in efforts to use stem cells as therapies. If successful, the disease-in-a-dish concept could speed up researchers' understanding of many different diseases and lead to faster, more efficient screening of potential drug therapies, because scientists can test potential drugs in these custom-made cultures for both therapeutic efficacy and toxicity. In addition to the ALS work, the induced stem cells are currently being used experimentally to model dozens of illnesses, including sickle cell anemia, many other blood disorders and Parkinson's disease. Researchers in Germany, for example, have created cardiac cells that beat irregularly, mimicking various heart arrhythmias. Pharmaceutical companies, long wary of stem cell science as a commercial enterprise, are starting to show greater interest because the disease-in-a-dish approach complements the traditional strengths of industrial drug discovery.

The first fruit of the ALS experiment was published in 2008. As in most cases of innovation, success depended not only on the soundness of the idea but on the right mix of people pursuing it. In this case, the cast of characters, in addition to Chung, included Lee L. Rubin, a refugee from the biotech industry who

IN BRIEF

Still waiting: Stem cells from embryos hold promise for treating incurable conditions; however, investigators have not so far made much progress in deriving therapies from stem cells.

A new idea: Rather than focusing on treatments, a few researchers think stem cells are better suited—for now—to help screen for drugs and to investigate how different diseases damage the body.

Creative approach: Until recently, the stem cells needed to pursue this idea were made using embryos. But in 2007 scientists managed to reprogram adult human cells into stem cells.

Customized stem cells: Researchers are using these reprogrammed cells to re-create various diseases in a petri dish. Then they can test potential drugs against the refashioned tissue samples.

became head of translational medicine at the Harvard Stem Cell Institute, and Kevin C. Eggan, a tireless young stem cell scientist from Harvard, who was collaborating with Christopher E. Henderson and other motor neuron experts at Columbia.

A NEW ROLE FOR STEM CELLS

THE STEM CELLS used in these studies should not be confused with embryonic stem cells—the kind derived from early embryos. A dozen years ago James A. Thomson and his colleagues at the University of Wisconsin–Madison electrified the world with the news that they had created human embryonic stem cells in a lab for the first time. These primordial cells had the biological endurance to renew themselves forever and the versatility to turn into any cell type in the body. The possibility of using stem cells to create made-to-order transplants for everything from Parkinson's to diabetes tantalized doctors, researchers, the public at large and, most of all, patients with incurable conditions.

But two harsh realities awaited. First, a loud public debate over the ethics of stem cell science politicized the science and slowed research; the technology posed moral questions because human embryos had to be destroyed to harvest the embryonic stem cells. That debate culminated in President George W. Bush's announcement in August 2001 that the National Institutes of Health would restrict funding support to research using only a few existing embryonic stem cell lines, which effectively impeded the generation of additional stem cells, including the disease-specific cell lines. In response, prominent research groups at Harvard, Columbia and Stanford universities, along with patient advocacy groups such as Project ALS and the New York Stem Cell Foundation, created separate, "nonpresidential" labs to pursue research with private funding. In 2009 the Obama administration relaxed the rules governing stem cell research, but a federal court ruling in 2010 banned grant support from the National Institutes of Health once again and plunged the field into scientific uncertainty and funding chaos.

The second problem was scientific. As Valerie Estess, scientific director of Project ALS, recalls it, there was a mad rush to test the idea that specialized cells derived from stem cells could simply be transplanted into sick people (or animals) as cellular therapies to cure a host of diseases. "The big dream," she explains, "was to derive motor neurons from stem cells, and then you would put them in the brain or spinal cord, and the patients would just get up and start dancing the Watusi." But it did not work out that way in repeated animal experiments. "From beginning to end," Estess says, "these experiments were failures."

In 2002 Thomas M. Jessell, Hynek Wichterle and their team at Columbia published a landmark paper in the journal *Cell,* spelling out the ingredients and procedure for nudging embryonic stem cells down a biological pathway to form motor neurons. One researcher who saw in that work promise for a different use of stem cells was Rubin. Elfin and enthusiastic, Rubin had trained in neuroscience and served as research and chief scientific officer of a Massachusetts biotech company called Curis. He realized that creating a disease in a dish offered a potentially revolutionary way to discover drugs. And unlike a lot of academic scientists, he knew something about drug discovery. During a previous stint in biotech, he worked on a molecule that ultimately became the billion-dollar multiple sclerosis drug Tysabri.

After hearing the results of Jessell and Wichterle's research, Rubin drafted a business plan for a new kind of stem cell institute, "one that focused," he says, "not on cell therapy—which all stem cell biologists were interested in—but on using stem cells to discover drugs." At the time, venture capitalists wanted nothing to do with the idea. So Rubin nursed the idea along at Curis, working on spinal muscular atrophy, a childhood motor neuron disease that has a similar pathology to ALS. When Curis decided to drop the project in 2006, he quit biotech and moved to the Harvard Stem Cell Institute to pursue the disease-in-a-dish idea.

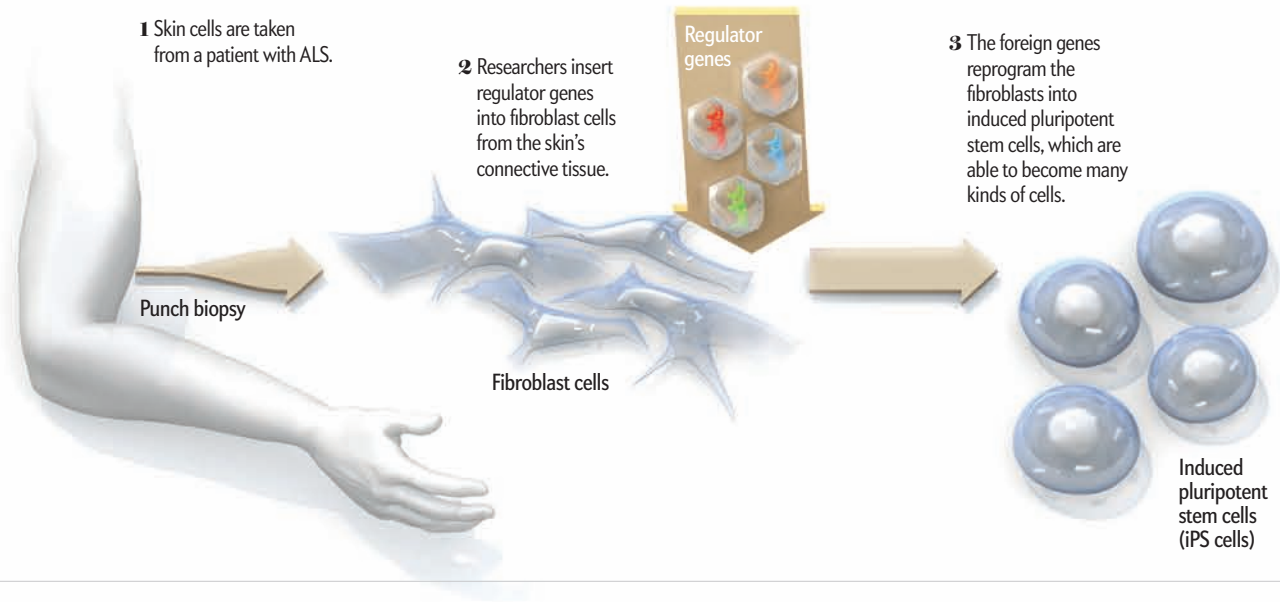
Shortly afterward, a Japanese biologist named Shinya Yamanaka disclosed a technique that would ultimately transform both stem cell biology and stem cell politics. At a scientific meeting at Whistler, B.C., in March 2006, the Kyoto University scientist described a procedure by which biologists could take ordinary adult mammalian cells and "reprogram" them. In essence, Yamanaka had biochemically reset the adult cells back to an embryoniclike or stemlike state without needing to use or destroy an embryo. He called the cells "induced pluripotent stem cells," or iPS cells. A year later both Yamanaka and Wisconsin's Thomson separately reported that they had created iPS cells from human tissue [see "Your Inner Healers," by Konrad Hochedlinger; SCIENTIFIC AMERICAN, May 2010].

One of the people sitting in the audience that day in Whistler

Cold storage: Biopsies and stem cells are preserved in liquid nitrogen.

New Uses for Old Skin

Using techniques pioneered in Japan, researchers from Harvard and Columbia universities extract skin tissue from adults (*below*), isolate specialized cells called fibroblasts from the sample, then gently coax them with genes and chemicals to become nerve cells.



was Eggan, who was a cellular reprogramming expert at Harvard. In fact, he had already embarked on his own version of the disease-in-a-dish idea, launching several projects to take an adult cell and biochemically coax it back into an embryolike state, allow it to replicate, and harvest stem cells from the resulting colony. He was trying to make embryolike cells the "old-fashioned" way, however, by applying the same cloning technique that produced Dolly the sheep. Eggan would take the nucleus out of an adult cell, such as a skin cell, and implant it into an unfertilized egg whose own nucleus had been removed. Cloning, however, was terribly inefficient and also terribly controversial if you planned to reprogram human cells—not least because you had to find women willing to donate their egg cells for the procedure.

Using Yamanaka's approach, however, Eggan and his team finally got the iPS technique to work in a test run with human cells in the summer of 2007. Everything else was already in place to try the disease-in-a-dish concept. Chung and her Columbia colleagues, for example, had collected cells from the two Croatian sisters and other ALS patients in anticipation that they would be used in Eggan's cloning experiments. With private funding, Project ALS had created a special laboratory near Columbia where researchers had been stockpiling cell lines from patients (including the elderly sisters) for months. Suddenly, the iPS approach offered a better chance of success. "That was complete kismet, that we had begun to collect human skin cells with a very different experiment in mind," says Estess of Project ALS.

The headliner among all those first ALS cell lines was the one from the younger, sicker Croatian sister, identified as patient A29. The skin cells of both sisters were successfully reprogrammed into nerve cells, but the age and degree of illness in patient A29 demonstrated that the iPS technique could be used to create cells that reflected a serious, lifelong disease. "We chose those samples because those were the oldest people in our study," Eggan says. "We wanted to prove the point that you could reprogram cells even from a very, very, very, very old person who'd been sick for some length of time. They were a special case."

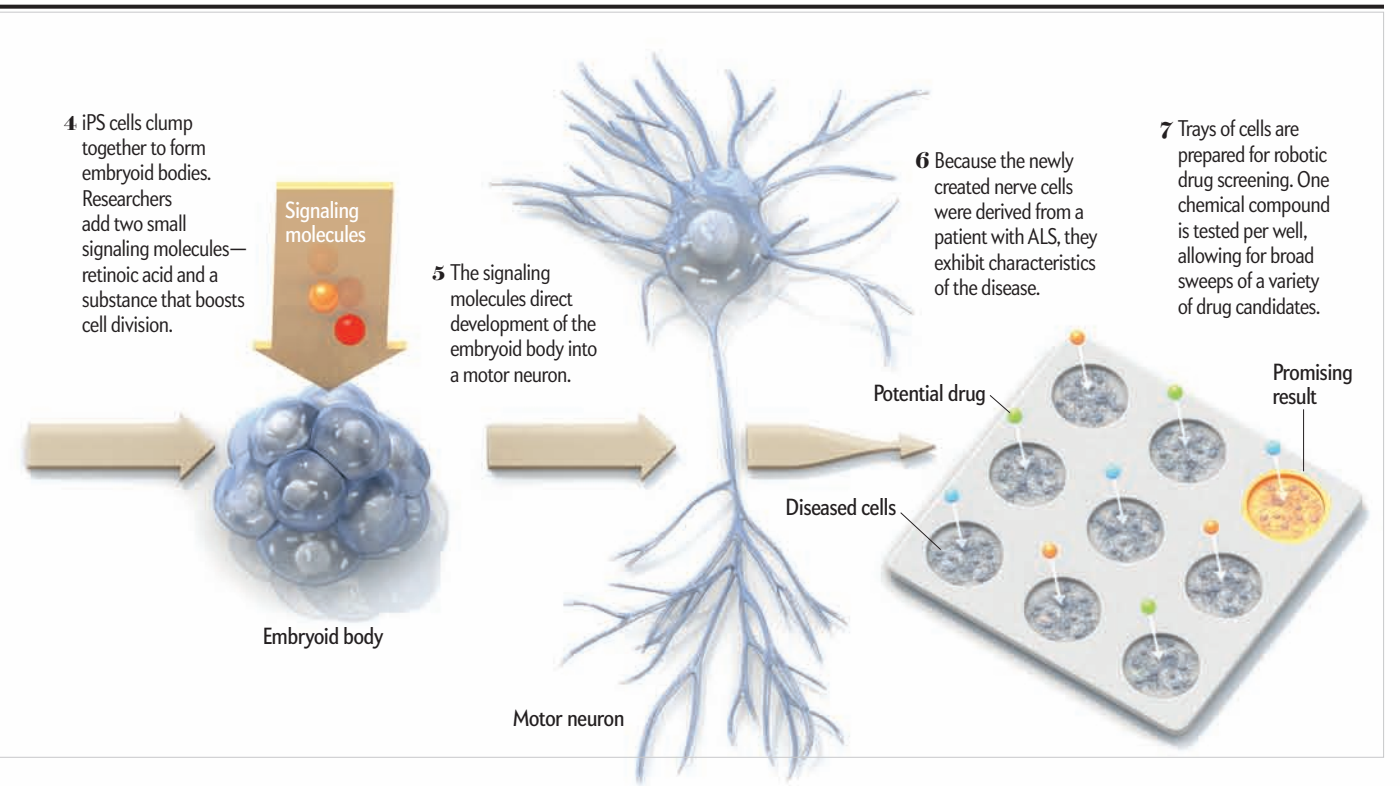
The results appeared in the August 29, 2008, issue of *Science* and were hailed in the press as a scientific milestone. The idea of using stem cells to create a disease in a dish promised experimental access to cells that were otherwise difficult or impossible to obtain—the motor neurons characteristic of ALS and spinal muscular atrophy, brain cells in many neurodegenerative disorders, and pancreatic cells typical of juvenile diabetes.

MADE-TO-ORDER STEM CELLS

IN THE PAST TWO YEARS the Columbia-Harvard collaboration has produced no fewer than 30 ALS-specific human cell lines, with more on the way. Many of these cell lines capture unique mutations found in people with unusually severe cases of ALS. More important, the disease-in-a-dish approach is beginning to deliver on its potential, providing insights into the nature of motor neuron disease. Using cells from the two sisters, for example, researchers have identified molecular pathways that seem to be involved in the death of motor neurons, which occurs when these cells are poisoned by another class of neurons known as astrocytes. With both motor neurons and astrocytes in a dish, scientists are now searching for potential therapeutic compounds that can either block the toxic activity of astrocytes or enhance the survival of motor neurons.

In January 2010, for example, researchers at the Project ALS lab began a preliminary screen of about 2,000 compounds in ALS motor neurons from humans, looking to see if any of the molecules would prolong the survival of nerve cells that contain the mutated ALS gene. This initial pilot program reflects a novel approach to drug screening: the ALS researchers began by testing compounds that have already been approved by the Food

Illustration by Bryan Christie



and Drug Administration for other illnesses. The hope is that researchers might get lucky and find a molecule, already tested and proved safe in humans, that could be rapidly repurposed for motor neuron disease. Pursuing a parallel track at Harvard, Rubin has identified almost two dozen small molecules that interact with one of the newly identified pathways and enhance the survival of motor neurons. The Spinal Muscular Atrophy Foundation is currently testing one of the molecules in an animal model of spinal muscular atrophy.

Perhaps an equally telling indicator that iPS cells offer a promising approach to drug discovery is the fact that Rubin is no longer banging his head against the door of pharmaceutical companies. Since the Columbia and Harvard researchers established the principle of a disease in a dish—that neurons with the genetic makeup of those in a diseased person can be produced—with patient A 29 in the summer of 2008, pharmaceutical companies have been banging on Rubin's door. Without naming specific companies for confidentiality reasons, he says, "I would say that of the major pharmaceutical companies, all of them have become interested in this approach now." The excitement has spilled over into biotech: many of the researchers in the motor neuron disease-in-a-dish story, including Eggan and Rubin, have become involved in a California-based biotechnology company called iPierian, which is one of several start-ups, including Cellular Dynamics International and Fate Therapeutics, that are adapting iPS technology for drug discovery.

MORE ON IPS CELLS
ScientificAmerican.com/mar2011/ips

Meanwhile more and more stem cell researchers are pursuing the disease-in-a-dish concept. Shortly after the ALS publication in 2008, a separate group of researchers at the Harvard Stem Cell Institute reported using the iPS technique to create disease-in-a-dish cells from patients with juvenile diabetes, Parkinson's and other disorders. And in late 2008 researchers at Wisconsin, led by Clive N. Svendsen (who has since moved to Cedars-Sinai Medical Center in Los Angeles), created motor neurons in a dish from a patient with spinal muscular atrophy.

When I asked researchers at Columbia and Harvard if the two Croatian sisters were aware of the research that grew out of their donated cells, no one seemed to know at first. But I eventually learned that the sisters are still alive, according to the daughter of patient A29, who agreed to speak as long as her name and those of family members remained anonymous. The older sister, now 93, remains essentially free of symptoms of ALS; indeed, according to her niece, she still "lives by herself, walks everywhere, shops, cooks, sweeps and cleans." The younger sister, patient A29, turned 85 last June; despite her ALS, she can move "slowly and weakly" and is "grateful" to have had the opportunity to help.

Still, the family's cruel burden never seems far away and underscores the urgency felt by those who might benefit from the new stem cell approach to finding drugs. "I am relatively young," says patient A29's daughter, who herself was diagnosed with ALS in 2002. "We are afraid that the onset of the disease is becoming earlier as the generations go along. We feel a little like"—she pauses as she speaks, to gather herself and her inevitably grim thoughts—"it's a race against time. I myself have a teenage daughter, and it just weighs so heavily on the mind and heart." SA

MORE TO EXPLORE

Induced Pluripotent Stem Cells Generated from Patients with ALS Can Be Differentiated into Motor Neurons. John T. Dimos et al. in *Science*, Vol. 321, pages 1218–1221; August 29, 2008.
Study Says Brain Trauma Can Mimic A.L.S. Alan Schwarz in *New York Times*, August 17, 2010.
iPS Cells: A Promising New Platform for Drug Discovery. George Daley in Children's Hospital Boston's science and clinical innovation blog, September 23, 2010: http://vectorblog.org/ips-cells-a-promising-new-platform-for-drug-discovery
Diseases in a Dish Take Off. Gretchen Vogel in *Science*, Vol. 330, pages 1172–1173; November 26, 2010.