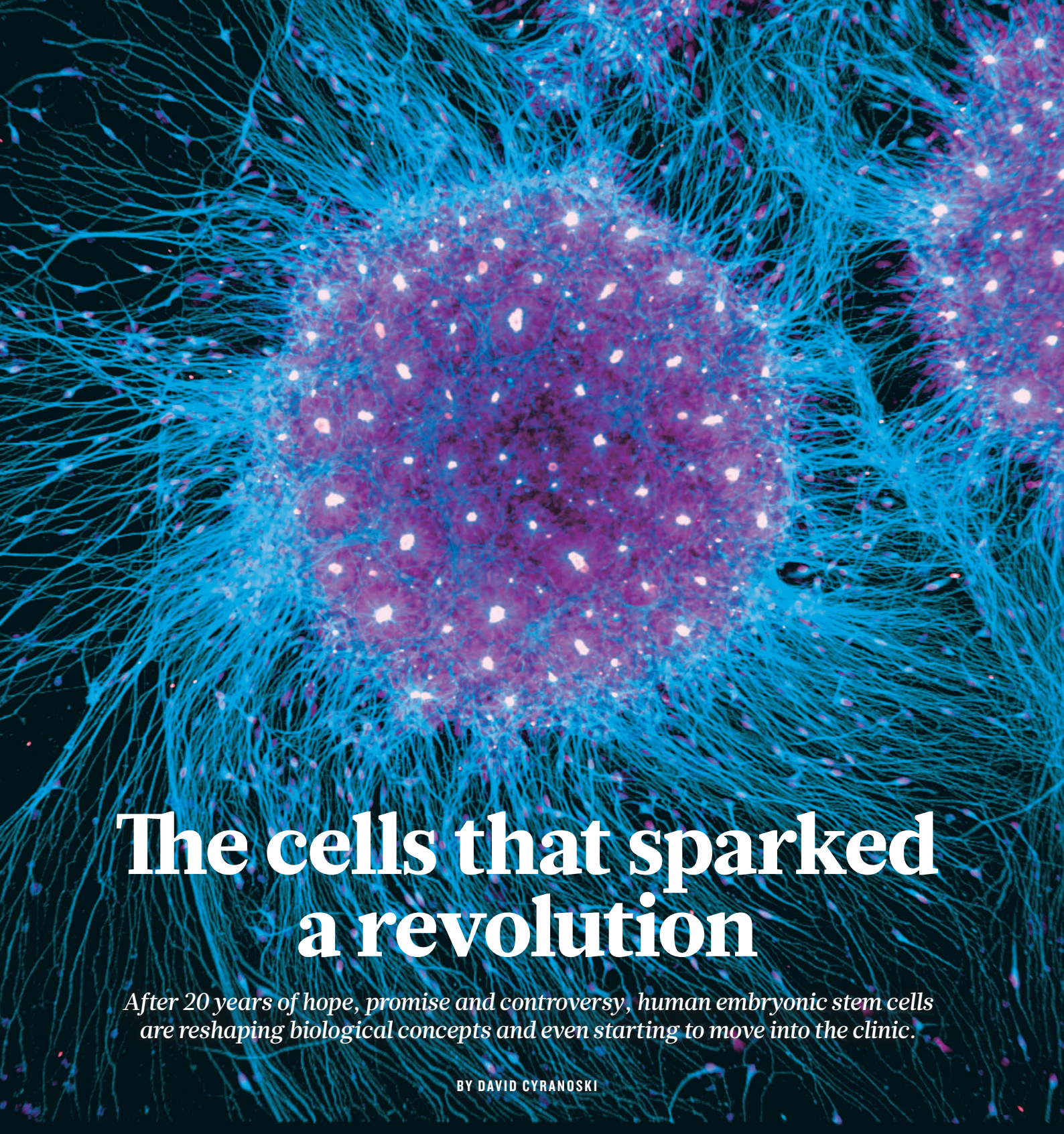

The cells that sparked a revolution

After 20 years of hope, promise and controversy, human embryonic stem cells are reshaping biological concepts and even starting to move into the clinic.

BY DAVID CYRANOSKI

Neural rosettes, derived from human embryonic stem cells, assemble into spheres in culture.

Dieter Egli was just about to start graduate school in 1998 when researchers first worked out how to derive human embryonic stem cells. In the two decades since, the prolific cells have been a fixture of his career. The biologist, now at Columbia University in New York City, has used them to explore how DNA from adult cells can be reprogrammed to an embryonic state, and to tackle questions about the development and treatment of diabetes. He has even helped to develop an entirely new form of human embryonic stem cell that could simplify studies on what different human genes do[1].

His wide-ranging research established him as a leader in embryonic stem-cell biology, a field challenged by restricted funding and an enthusiasm for competing technologies that don't carry the same ethical baggage. Still, many say that human embryonic stem cells are now more relevant than ever. "I am very excited about embryonic stem cells," says Egli. "They will lead to unprecedented discoveries that will transform life. I have no doubt about it."

Embryonic stem (ES) cells provide unparallelled information on early development. Like astronomers looking back to the Big Bang

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for fundamental insight about the Universe, biologists rake over the molecules inside these remarkable entities for clues as to how a single original cell turns into trillions, with a dizzying array of forms and functions. Scientists have learnt how to turn the cells into dozens of mature cell types representing various tissues and organs in the body. These are used to test drugs, to model disease and, increasingly, as therapies injected into the body. Starting with an attempt to repair spinal-cord injuries in 2010, there have been more than a dozen clinical trials of cells created from ES cells — to treat Parkinson's disease and diabetes, among other conditions. Early results suggest that some approaches are working: a long-awaited report this week shows improved vision in two people with age-related macular degeneration, a disease that destroys the sharpness of vision[2].

"In some ways, it's not a surprise, because 20 years ago we expected it," says Egli, "but I'm still surprised that this promise is becoming a reality."

TENTATIVE BEGINNINGS

In 1981, researchers managed to culture stem cells from mouse embryos. They soon recognized the research potential of these intriguing entities, which can both replicate themselves and be nudged into becoming any of the body's 200-plus cell types[3,4]. But that trick was not easy to accomplish in primates. It took biologist James Thomson, at the University of Wisconsin–Madison 14 years to achieve it in monkeys[5]. Three years later, using donated embryos that had gone unused in fertility treatments, Thomson struck again, creating the world's first human ES-cell line[6].

The discovery sparked an ethical firestorm. Critics, mostly from religious circles, argued that embryos constitute human beings, and wanted to prevent any research that involved destroying them. In 2001, US President George W. Bush restricted government funding to research on just a few existing ES-cell lines. The decision effectively forced those intent on carrying out the research in the United States to seek private or state funding, and often to create duplicate laboratories — one for ES-cell research and another for work funded by the US federal government. In other countries, including Germany and Italy, the creation of the cells was banned altogether.

Nonetheless, the research went forward where it could. Investigators in Australia, Singapore, Israel, Canada and the United States, among others, soon reported that they had converted embryonic stem cells into neurons, immune cells and beating heart cells[7].

Researchers also discussed plans to derive stem cells from embryos made by a process called somatic-cell nuclear transfer — the same method used to create cloned animals such as Dolly the sheep — in which the nucleus from an adult donor cell is transferred into a human egg that has had its nucleus removed. The rationale for this 'therapeutic cloning' was to provide a limitless source of dynamic cells with the same DNA as the cell donor. They started talking about studying complex genetic diseases 'in a dish' and replacing failing organs and tissues in the same way that mechanics replace car parts. There were several false starts, notably in 2005, when investigators found that South Korean scientist Woo Suk Hwang had fraudulently claimed to have isolated stem cells in this way. But by 2013, a team led by Shoukhrat Mitalipov, a stem-cell researcher at the Oregon Health and Science University in Portland, finally succeeded[8].

> *"In some ways, it's not a surprise, because 20 years ago we expected it."*

Throughout the first 15 years, however, much ES-cell research focused on using the cells to understand pluripotency — the amazing ability to become any type of cell. Bit by bit, scientists have been piecing together the molecular pathways that make it possible. "We learned pluripotency from ES cells," says Mitalipov.

Such research contributed to arguably the biggest innovation in regenerative medicine and biological research in the 2000s: the discovery of induced pluripotent stem (iPS) cells. In 2006, stem-cell biologist Shinya Yamanaka at Japan's Kyoto University worked out how to return adult mouse cells to an embryonic-like state using just four genetic factors[9]. The following year, he and Thomson achieved the same feat in human cells[10,11]. The process offered, in theory, the same potential payoff as therapeutic cloning — a limitless supply of pluripotent cells that are genetically matched to a patient — but without the ethical quandaries.

Many predicted that iPS cells would soon displace embryonic stem cells in the research space, but it didn't happen. The number of ES-cell publications grew rapidly after 2006 and has held pace, at about 2,000 per year since 2012. Part of the reason was that ES cells were the gold standard against which researchers could compare iPS cells. And even today, there are some who doubt the safety of using iPS cells. Zhou Qi, a stem-cell biologist at the Chinese Academy of Sciences Institute of Zoology in Beijing, says that concerns that iPS cells would cause tumours inspired him to use ES cells for more than a dozen clinical trials he is orchestrating.

Much of the research on ES cells has been in making them easier to work with. Deriving them was initially a finicky process: plucking one from a culture and growing it into a new population worked less than 1% of the time. A handful of advances has changed that. In 2007, for example, Yoshiki Sasai at the RIKEN Centre for Developmental Biology in Kobe, Japan, discovered a molecule, called a ROCK inhibitor[12], that could keep ES cells from dying when they were removed from the colonies in which they thrived. The success rate for creating new colonies shot to 27%. "It fundamentally changed what you could do," says cell biologist Malin Parmar at Lund University in Sweden. Parmar, who is using ES cells to derive neurons for a Parkinson's disease clinical trial, says that such technical advances ushered in "a new golden age" for ES-cell research.

The cells can now be produced quickly, reliably and indefinitely. And yet they somehow avoid turning into cancer, as some feared they would. "We still do not know why or how" they maintain this equilibrium, says Hiromitsu Nakauchi, a stem-cell biologist at the University of Tokyo, who has been trying to make unlimited supplies of blood platelets from ES and iPS cells.

TIME TO DIVERSIFY

Researchers are also trying to grow organs. Given the right signalling molecules and 3D environment, ES cells organize into complex tissues known as organoids, even in a dish. This capacity is important for researchers such as James Wells at Cincinnati Children's Hospital in Ohio, who is developing intestinal organoids for testing drugs, and perhaps one day for transplant.

And new sources of ES cells have presented other research tools for genetic disease. In 2004, for example, fertility doctors in Chicago started making ES-cell lines from embryos created through *in vitro* fertilization that had been found to have a genetic defect, and thus were rejected for fertility treatments. This allowed the team to create cellular models of thalassaemia, Huntington's disease, Marfan's syndrome, muscular dystrophy and other genetic conditions[13]. In 2007, researchers used ES cells to pin down the molecular changes that lead to cognitive impairments seen in a heritable condition known as Fragile X syndrome[14].

Researchers say that iPS cells promise even more for disease-in-a-dish studies — namely the ability to grow stem cells from any living person with a suspected genetic condition. But many investigators still see strong potential for ES cells in this area. Some conditions cause damage to adult cells that would make any iPS cells derived from them uninformative. And ES cells still have a supporting role.

In 2008, for example, Kevin Eggan at Harvard University in Cambridge, Massachusetts, produced iPS cell lines from people with the neurodegenerative disease amyotrophic lateral sclerosis (ALS). From previous work with ES cells, Eggan knew how to coax pluripotent cells into becoming motor neurons, the brain cells affected by the disease. When he did the

same with patient-derived iPS cells, he was able to quickly compare the two types of cell. Cells from patients fired much more than their counterparts from people without the disease[15]. "We took advantage of all the work we had done with ES cells to understand motor neurons," says Eggan. Now, an anti-seizure medicine that quieted iPS cells made from patients is being tested in humans. Results are expected in the next two months.

Egli and Nissim Benvenisty at the Hebrew University of Jerusalem overturned long-held concepts of human biology when they derived ES-cell lines with just half the normal number of chromosomes[1]. Researchers are now starting to use gene-editing tools on these 'haploid' ES cells to understand how genes function in development. Because they have only one set of genes to worry about, the cells could deliver much more straightforward results, Egli says.

The advances in disease research with ES cells have not all come smoothly. It took Douglas Melton at the Harvard Stem Cell Institute in Cambridge 15 years to turn ES cells into functional β-cells — the pancreatic cells that can sense glucose and produce insulin. Then he couldn't find any difference between pancreatic cells produced from normal ES cells and iPS cells from people with type 1 or 2 diabetes. "We know there is a genetic susceptibility, but that doesn't mean you can see it *in vitro*," he says.

> *"We took advantage of all the work we had done with ES cells to understand motor neurons."*

CELL REVIVAL

Melton still has plans for the β-cells he's made from ES cells. He hopes to transplant them into people with type 1 diabetes to end, or at least reduce, their reliance on insulin injections. The last hurdle in the work is introducing the cells so that they are not destroyed by the immune system. Semma Therapeutics, a company that Melton founded in Cambridge, aims to do this by ensconcing the cells in a pouch that would allow nutrients in and insulin out, but would block access to immune cells. He expects to start clinical trials within three years. ViaCyte in San Diego, California, has just restarted a similar clinical trial it launched in 2014 after redesigning its encapsulation technology. And other companies, such as Novo Nordisk in Denmark are starting up programmes for diabetes using cells derived from ES cells.

In the clinical realm, many have assumed that iPS cells would eventually win out over ES cells. One potential advantage is that they can produce cells and tissues with the same DNA as the patient and thus not cause an immune reaction when transplanted. But for most genetic diseases, including type 1 diabetes, iPS cells created from a patient would contain the mutation that causes the problem, and the cells would have to be modified to confer any therapeutic benefit.

Then there's the matter of cost. Preparing a single iPS-cell line for clinical use would cost roughly US$1 million, says Jeanne Loring, a stem-cell biologist at the Scripps Research Institute in La Jolla, California. That's currently prohibitive if the goal is to use a patient's own cells, but Loring expects that the price will come down and is working on developing iPS cells as a treatment for Parkinson's disease.

So far, researchers have initiated just one human trial using cells derived from iPS cells. Led by ophthalmologist Masayo Takahashi at the RIKEN Center for Developmental Biology, it aims to treat macular degeneration, but was halted in 2014 when investigators decided to simplify the procedure and use donor-derived, rather than patient-derived, stem cells. It restarted in 2017, but hit another roadblock in January, when a membrane developed in the eye of a participant and had to be surgically removed.

Macular degeneration has been a popular target for ES-cell therapies. There have been at least six clinical trials, in the United States, the United Kingdom, South Korea, China and Israel. On 19 March, researchers led by ophthalmologist Pete Coffey, director of the London Project to Cure Blindness and the University of California, Santa Barbara, reported the results of a study to implant a patch of cells made from ES cells into the damaged retinas of two individuals[2]. A year after the procedure, the participants regained the ability to read, albeit slowly.

Alan Marmorstein, an ophthalmologist at Mayo Clinic in Rochester, Minnesota, calls it a "big step forward" for the field. "This is the first strong indication of efficacy in humans and it certainly supports further studies in other parts of the body," he says. Coffey says the breakthroughs are finally arriving because scientists are now working out how best to put the cells into people. "A decade ago, we thought, 'You just needed to put the cells in, and the cells will know what to do.' That's not true — they have to be controlled in some appropriate way." Many in the stem-cell field are betting the next big clinical breakthrough for ES cells will come in Parkinson's disease. The disorder is caused by a loss of the neurotransmitter dopamine, and half a dozen companies and clinics are gearing up to use ES cells or iPS cells to replace dopamine-producing neurons.

One crucial question is how far the pluripotent cells should be taken down the road towards maturity before transplanting them. An Australian trial started in 2016 and a Chinese trial begun in 2017 use immature neural precursor cells, which do not produce dopamine. The researchers say the immaturity of the cells will help them to survive transplantation and integrate into their new host's brain. But leaders of a group of ES- and iPS-cell trials known collectively as GForce-PD say that the more-mature cells they use turn into the desired type of dopamine-producing cell more reliably and are less likely to grow out of control.

PATHWAY TO PROMISE

ES-cell research still has room to grow, if it can get past some hurdles. One big problem is that many cell types are challenging to produce. Melton estimates that only about ten cell types created so far are truly functional equivalents of normal human cells. And some with the most far-reaching uses, such as eggs and sperm, are expected to remain a challenge for the foreseeable future.

The field also faces uncertainty about funding. Scientists have heard frequent rumours that US president Donald Trump might impose new restrictions on federal funding for research on ES cells.

But despite their sometimes rocky history, ES cells have proved their value repeatedly, and in some unpredictable ways, say many investigators. Some researchers have even scaled back their use of animal models because ES cells seem to provide a better path to studying human disease. "My motto was, 'all human, all the time'," Melton says.

Yamanaka says that ES cells were the motivation for his own work on iPS cells. And it was Thomson's recipe for human ES cells that allowed the shift from mouse to human iPS cells in just one year, after it had taken nearly two decades to move from mouse ES cells to the human variety. "We knew exactly how we should culture human iPS cells," says Yamanaka.

ES cells are just as crucial today, he says, for better understanding the mechanism of pluripotency and for improving the medical application of any pluripotent cell. "The importance of human ES cells is no less now than 20 years ago, and I do not imagine it will be any lower in the future," he says. ■

David Cyranoski *writes for* Nature *from Shanghai, China.*

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