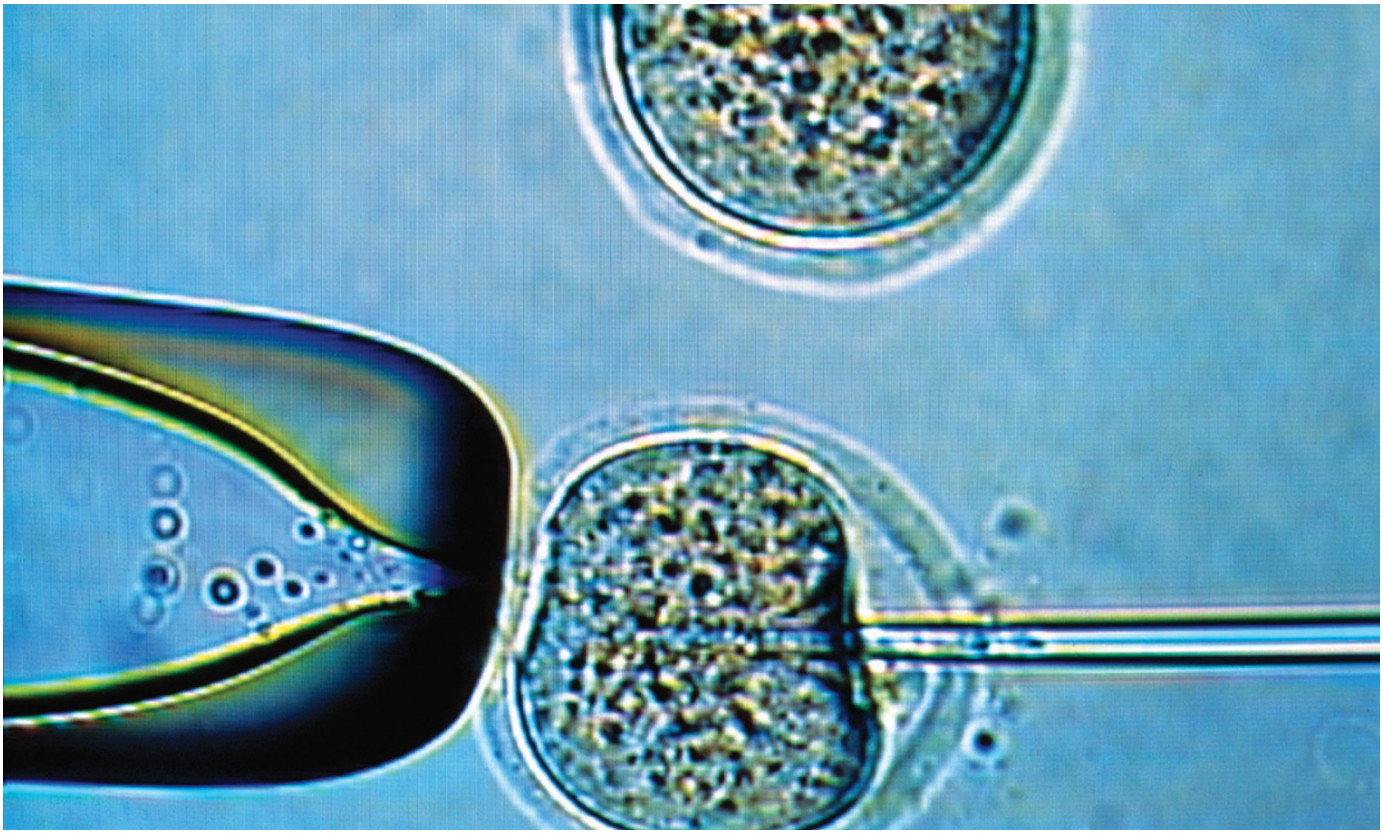

JAMES KING-HOLMES/SCIENCE PHOTO LIBRARY

A mouse embryo is held in place during nuclear transfer. The egg's genetic material has been removed and an adult cell nucleus is injected in its place.

STEM CELLS

Flexible friends

Stem cells are powerful tools in biology and medicine. What can scientists do with these cells to exploit their incredible potential?

BY NIRUPAMA SHEVDE

At first, people and objects seem fuzzy around the edges. Faces become unrecognizable in low light. Reading and driving are no longer possible. Eventually, darkness descends.

A disease called macular degeneration is responsible for this progressive loss of vision. The light-sensitive cells in the eye, located in a thin layer of tissue called the retina, are damaged and stop working. Macular degeneration is mainly a disease of ageing and is the leading cause of vision loss in people aged 65 and older. It is expected to affect nearly 3 million people in the United States by 2020.

Related diseases, including a genetic condition called Stargardt's macular dystrophy, affect young people as well. With this condition, fatty deposits build up behind the retina, causing it to degenerate, and vision loss is inevitable. What if those dying cells in the retina could be repaired or replaced? Scientists are testing a daring new treatment for both macular degeneration and macular dystrophy. They are injecting replacement cells into the back of the eye to repair the retina. The company running the trials, Advanced Cell Technology, makes the replacement eye cells from human embryonic stem cells.

Stem cells are powerful. They are pluripotent, which means they can transform into any one of the 220 cell types in the human body. One stem cell can also divide to produce millions more stem cells. The potential for stem cells to renew themselves, or to create new tissue, is almost infinite. These properties make stem cells an important tool in the lab and in medicine. They offer scientists a better understanding of human development and a way to test drugs without putting human volunteers at risk, and provide a way of replacing damaged tissues, such as the cells of the retina, muscle or spinal cord. But how can we make the best use of stem cells? How can we make them grow where we want, grow how we want, and repair damaged or diseased tissue?

To understand stem cells, we first must establish their basic biology. There are stem cells in nearly all animals, from tiny worms to mice and humans. There are three types of stem cell — embryonic stem cells, adult stem cells, and induced pluripotent stem (iPS) cells — each with its own capabilities and limitations. An embryonic stem cell comes from an organism at its earliest stages of development. When a sperm meets an egg and the resulting zygote begins to divide, each cell holds the potential to become any cell type in the body. This is the essence of pluripotency. Not until the cells divide a few times do they start to lean towards one fate or another, expressing genes specific to one cell type. This process is called differentiation.

Once an embryo has developed into a mature organism, most cells have undergone differentiation, but some cells are special. Unlike most adult cells, they retain the ability to multiply and become other types of cell. These adult stem cells reside in special stem-cell niches, regions of certain tissues where they wait for cues from

the organism to replace or repair tissue. They are usually found in tissues that must continuously replenish themselves, such as the blood, the skin and the gut. But they have also been found in the brain, which replaces its cells much less frequently. These adult stem cells are said to be multipotent — unlike pluripotent cells, they cannot turn into any one of the 220 cell types in the human body. Neural stem cells in the brain can differentiate into several kinds of brain cell but could not become liver cells, for example.

In the past decade, scientists have also learned to make stem cells from regular mature, differentiated cells. These cells are called induced pluripotent stem cells because scientists force them to become pluripotent even after they have reached a differentiated state. By turning up the expression of just a few genes, scientists can force a skin cell, for example, to retrace its developmental pathway backwards all the way to a flexible pluripotent state.

The ability to manipulate the fate of a cell has led to much excitement about the potential of these fascinating cells. But stem-cell research is an emerging field, with many fundamental questions still unanswered. For example, how do the three types of stem cells differ, and how are they the same? Can we use these cells to cure diseases? And can we use them to rebuild tissues or organs?

AN EYE ON DEVELOPMENT

Every cell in the body has the same set of genes. An eye cell is different from a liver cell because they differ in which genes are turned on and which genes are turned off. In a retinal cell, genes that enable light sensing are turned on, and genes that make digestive proteins are turned off. In liver cells, the opposite is true. The scientists who are using embryonic stem cells to cure blindness have figured out which genes are on and which genes are off in the retinal cells that patients need. By growing pluripotent embryonic stem cells with chemicals and proteins that make them differentiate into retinal cells, the researchers have an unlimited supply.

The ability to do this is the result of decades of research, going back to the discovery of stem cells. In 1868, German biologist Ernst Haeckel first used the term 'stem cell' to describe a zygote. Researchers have been working with mouse embryonic stem cells since 1981 but the field took off in 1998, when researchers isolated human embryonic stem cells for the first time[1].

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The breakthrough came when James Thomson and his team at the University of Wisconsin–Madison obtained a group of cells called an 'inner cell mass' from a blastocyst, which is a natural source of stem cells. A blastocyst is a very early stage embryo, created here from the controlled fertilization of an egg by a sperm in the laboratory. It is simply a hollow ball of cells with a cluster of cells inside. Scientists at fertility clinics often make more embryos than prospective parents will need to get pregnant, and the surplus embryos are usually frozen or discarded, according to the parents' wishes. In this case, the parents offered their surplus blastocysts to science. If the blastocysts were implanted into a uterus, the inner cell mass would become a fetus and the outer shell, or trophectoderm, would form accessory tissues such as the placenta. Without implantation, however, the blastocysts can be converted to cell cultures.

To do this, the researchers removed the inner cell mass and grew the cells in a flat dish create a cell line, a set of genetically identical cells. This required a nutrient-rich liquid and some mouse cells to serve as feeder or support cells.

The isolated cells grew and grew. Indeed, part of the definition of a stem cell is that it can divide almost infinitely, producing more and more stem cells. In contrast, ordinary cells die after a certain number of divisions, limited by telomeres, or caps on the DNA. The cell's DNA replication machinery cannot reach the very tips of the strand, so the telomeres shorten a bit with every cell division. Stem cells, however, have an enzyme called telomerase that builds the telomeres back up, essentially making the cells immortal.

But there is more to being a stem cell than being able to divide forever; the cells must also be pluripotent, having the capacity to become any type of cell. During embryonic development, all the body's organs and tissues develop from one of the three germ layers: endoderm, mesoderm and ectoderm. A pluripotent cell can form all three germ layers. A cell that can develop only one germ layer has already started down the road toward differentiation and is not pluripotent.

Scientists often test for pluripotency by injecting the cells into a mouse. Once in the mouse, pluripotent cells will form a teratoma, a clump of cells containing all three germ layers. Thomson's cells passed the test. The researchers had discovered how to produce embryonic stem cells.

Scientists now have a supply of embryonic stem cells that will divide indefinitely and are pluripotent. They know how to isolate them and work with them in the lab, and they know how to keep them healthy in the correct culture conditions. So why haven't they already turned embryonic stem cells into every type of cell they need to cure disease?

Unfortunately, it's not that easy. In the case of the retina cells needed to cure macular degeneration, researchers have discovered which molecules can coax stem cells to become the correct cell type for transplantation into the eye. However, they have not yet discovered the specific conditions and transplant techniques for all 220 of the cell types in our bodies. Different cell types require different conditions and molecular cues. As well as the technical limitations regarding the cell culture, scientists are held back by the human culture outside the lab because not everyone supports research on embryonic stem cells.

OBJECTIONS TO STEM CELL REASEARCH

What concerns do people have about the use of embryonic stem cells? The main objections are based on people's cultural and religious beliefs.

Catholics and some conservative Protestants, for example, oppose stem-cell research because they believe that human life starts at conception, making it unethical to destroy a zygote or an embryo, or even a blastocyst. People from other religions, including Judaism, Islam and Hinduism, view the embryo differently and are generally not opposed to stem-cell research. Still others object to the research because they are misinformed about the source of stem cells, believing they come from aborted human fetuses rather than blastocysts that have never been implanted. In addition, many scientists working on stem-cell research also hold strong religious beliefs, so it is not as simple as science on one side and religious beliefs on the other.

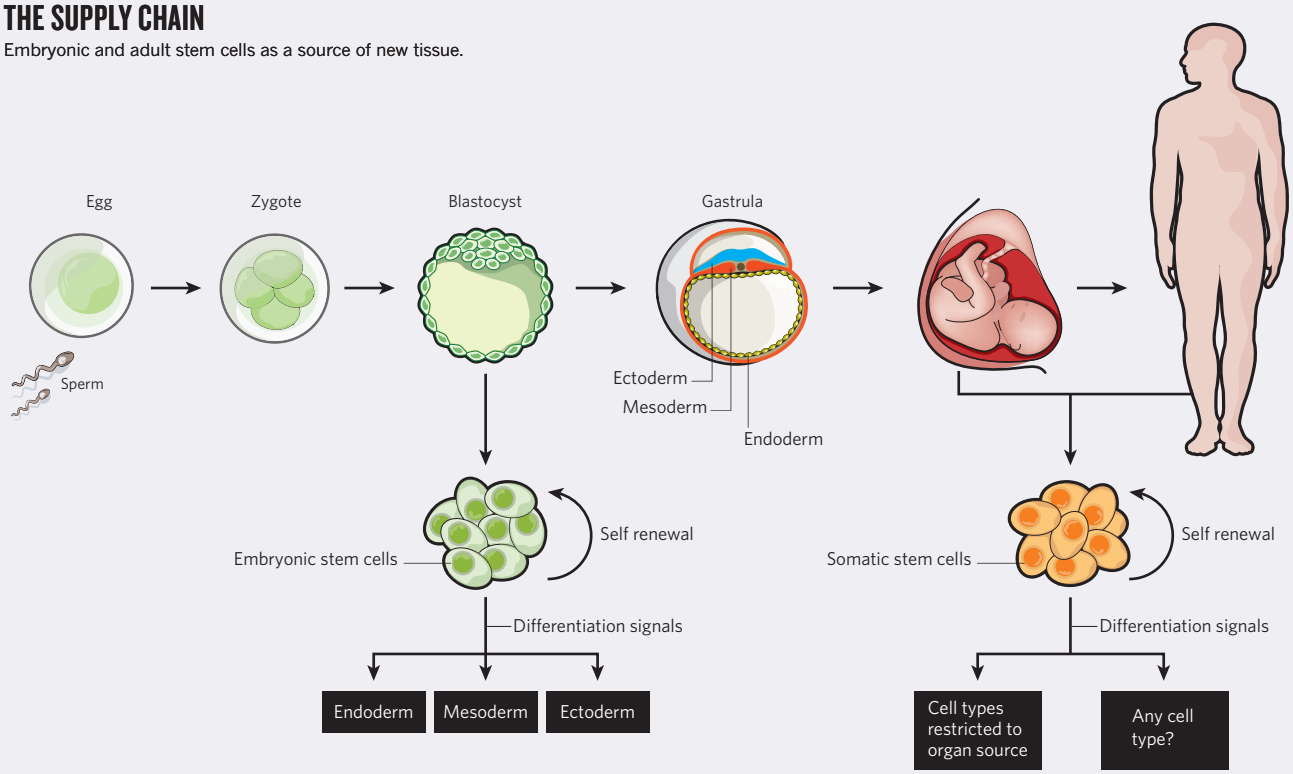
The research guidelines for stem-cell research in the United States are set by the US government, which funds most of the country's medical research. Not surprisingly, its view on embryonic stem-cell research alters with changes in presidential administrations. In 2001, President George W. Bush authorized the federal support of human embryonic stem-cell research only if the cells were derived before 9 August 2001 from an embryo that was created for reproductive purposes and was no longer needed. In addition, people donating embryos for research had to give informed consent by signing a document saying they know what the embryos would be used for, and they could not receive any money in return.

These restrictions limited the availability of human embryonic stem-cell lines to fewer than a hundred. In 2009, President Barack Obama issued an executive order so the federal government could support and conduct responsible, scientifically worthy human stem-cell research, including embryonic stem-cell research, to the extent permitted by law. Even so, fewer than 200 cell lines are available for federally funded research. Scientists who need cell types not included in the federal registry must seek funding from states or private foundations. The Stanford Encyclopedia of Philosophy has published a thorough review of the ethical questions raised by stem-cell research, and the website of the US National Institutes of Health (http://stemcells.nih.gov/info/ethics.asp) offers several resources on the topic.

For a long time, scientists thought that once a cell differentiated, it could never regain its pluripotency. They assumed that it must be set in its identity because it had so many genes turned on and off in ways particular to that cell type. In 2006, however, Kazutoshi Takahashi and Shinya Yamanaka of Kyoto University in Japan proved otherwise. They turned

THE SUPPLY CHAIN

Embryonic and adult stem cells as a source of new tissue.



mouse skin cells — specifically, fibroblasts — into stem cells. These were the first induced pluripotent stem (iPS) cells.

How do we rewrite a cell's developmental programming? The researchers thought that if they could force a cell to turn on the genes that are turned on in stem cells, and turn off all the other genes, that cell would become a stem cell. So they needed to know which genes are crucial to the stem-cell phenotype. What genes make a stem cell a stem cell?

The scientists selected 24 genes that they thought might contribute to a stem cell's pluripotent nature. They turned on these genes in the mouse fibroblasts, in many different combinations. In the end, they found that a mix of four genes that encode transcription factors — molecules that turn genes on and off — could turn fibroblasts into iPS cells. The genes were *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*.

Yamanaka and colleagues still needed to prove that their iPS cells were fully pluripotent, and that meant showing they could form an entire animal the way embryonic stem cells do. They added some of their iPS cells to a developing mouse embryo and showed that the iPS-derived cells formed part of the resulting mouse. Two other teams published similar results in the same week.

The iPS cell research race was on, and although Yamanaka had started it, his group soon had to keep pace with a crowd of other scientists excited by his discovery. Several investigators are now reprogramming cells to make their own iPS cells. Harvard University and other universities have entire centres devoted to iPS studies. One of the great benefits of iPS cells is that they do not need donated embryos.

Scientific progress often accelerates when several groups are exploring the same question. The stress to make an important discovery and publish first can be overwhelming, but that kind of motivation can spur a field on. Different research groups can confirm one another's results, build a case for well-supported theories, and quickly expose incorrect hypotheses.

The next immediate question was whether iPS cells can be made from human tissue. In 2007, three papers published in rapid succession reported the making of human iPS cells. Yamanaka and one other group used the original recipe[2], whereas a third research team, led by Thomson, used a different technique.

Progress was rapid. It took less than six months for scientists to modify the mouse iPS protocol to work for human cells. In contrast, it had taken 17 years to move from the first mouse embryonic stem cell to the first human embryonic stem cell, obtained by Thomson in 1998.

Now that they knew how to make human iPS cells, researchers wanted to improve the process. Some parts of the methods might be dangerous to patients who might one day be candidates for stem-cell therapies. The two main concerns were genes that can cause cancer and the use of viruses to deliver these genes via viral vectors. One of the genes that Yamanaka and others used to make iPS cells was c-Myc, a cancer-causing oncogene. Mice made from iPS cells that have high levels of the c-Myc protein frequently develop tumours, probably because c-Myc encourages not only stem-cell production, but also cancerous growth. Because of the similarities between stem cells and cancer cells, the possibility that stem cells could turn cancerous is a major concern for scientists.

In 2008, Yamanaka and his colleagues announced that they had managed to eliminate c-Myc from their recipe, rather by accident. In a series of experiments, they discovered that Myc is not essential but merely speeds up the process, so their technique could work without it.

Some of the early iPS methods, including one developed by Thomson and his team, relied on viruses to carry the newly created DNA into the human cells. Could these viruses have a negative effect effect by activating cancer genes or causing some other undesirable gene expression? Some research groups have sidestepped this concern and developed virus-free techniques. For example, in 2008, the Kyoto researchers showed that they could move stem-cell genes into human genes without viruses by using plasmids, circular DNA molecules that can hop between cells and into genes. Like viruses, however, plasmids might also enter the genome at the wrong place, accidentally activating cancer genes. To avoid this risk, researchers later created yet another method for delivering stem-cell genes. They first inserted stem-cell genes into cells using non-integrating vectors or plasmids that can be cleaned out or removed once the cells had become pluripotent. This change eliminated the risk imposed by inserting a nucleic acid or plasmid[3].

It would be better still to get rid of the

gene-insertion technique altogether and rely on chemical factors in the growth medium to force cells towards the pluripotent state by changes in the transcription of their own genome. So far, researchers working with mice have managed to replace the signals normally activated by transcription factors with chemical signals. Amazingly, they have replaced everything except the effects of transcription factor Oct3/4.

CELL MEMORY

Just like embryonic stem cells, iPS cells can divide indefinitely and differentiate into the three germ layers. Unlike embryonic stem cells, however, iPS cells used to be differentiated cells. Might they retain some genetic or cellular 'memory' of their previous life as a skin cell or a muscle cell? Recent studies suggest that they do, in the form of epigenetic markers on the DNA. These markers are methyl, acetyl and other chemical groups that attach to the DNA, turning some genes off and others on. Cells acquire epigenetic markers as they differentiate, and they maintain some of them when they dedifferentiate into iPS cells.

The discovery that iPS cells retain some memory was serendipitous. George Daley of Harvard University noticed that he had more success making blood cells from iPS cells that used to be blood cells than with iPS cells that started out as skin cells. Daley's team and other groups found that iPS cells keep some of the epigenetic markers that turned genes on or off in the original, differentiated cells. How can we control these markers? Only when the markers are gone will scientists truly be able to say that iPS cells are no different to embryonic stem cells.

Many of the intended uses of stem cells involve transplanting them into patients. But concerns about the risk from cancer-causing genes leave some scientists wondering if they should bother with the iPS stage at all. If doctors could avoid a pluripotent step in their treatment, it would be less risky. Ideally, they would like to avoid removing and transplanting cells at all. Is it possible to use drugs to trigger someone's own cells to redifferentiate into the cell type they need?

This idea is starting to look plausible. Scientists have recently shown that it is possible to skip directly from one differentiated cell type to another. This approach, called transdifferentiation, is very appealing, and many scientists are working on it.

In 2008, Qiao (Joe) Zhou and Douglas Melton at Harvard University managed to turn one type of pancreatic cell in mice into another, the beta-islet cells of the pancreas that produce insulin. These are the cells that are destroyed in people with type 1 diabetes. Performing the process inside the animal is advantageous because the new cells can develop in their natural environment instead of in a lab dish.

Despite its medical significance, changing one pancreatic cell type to another is a fairly small jump between similar cell fates. Researchers led by Marius Wernig at Stanford University made a bigger leap in 2010 with a protocol to turn mouse fibroblasts into neurons. A year later, they accomplished the same feat with human cells. The neurons they made, however, are not exactly the same as any particular kind of brain cell; instead, they had a mixture of neural characteristics. In the same year, another group of scientists managed to transdifferentiate fibroblasts into the specific type of neuron that produces the neurotransmitter dopamine. Dopamine's functions include controlling movements, and these neurons are lost in people who have Parkinson's disease.

Researchers are still not sure whether the cells change directly from one type to the next, or whether they go through other, less-differentiated stages. And there is still no tried-and-true, reproducible method. More work is needed to make these cells useful for detailed studies or medical use.

ADULT STEM CELLS

Researchers are also trying to take advantage of the adult stem cells we all have in our bodies. These adult stem cells do not raise the ethical questions that embryonic stem cells do because no embryos are needed. The problem is that adult stem cells exist in very small numbers and are often buried deep in the tissue. Progress in this area has been hindered by lack of sufficient cells, but doctors have been using adult stem cells to help patients for about 40 years. They are mainly used for two treatments: bone-marrow transplants to rebuild a patient's immune system, and skin grafts to replace skin over a burn or other injury.

Scientists would like to develop other applications for adult stem cells. However, they still do not fully understand where adult stem cells come from and how they differentiate when needed. If we can solve these mysteries, doctors might be able to use drugs to activate a patient's own adult stem cells to perform the necessary repairs.

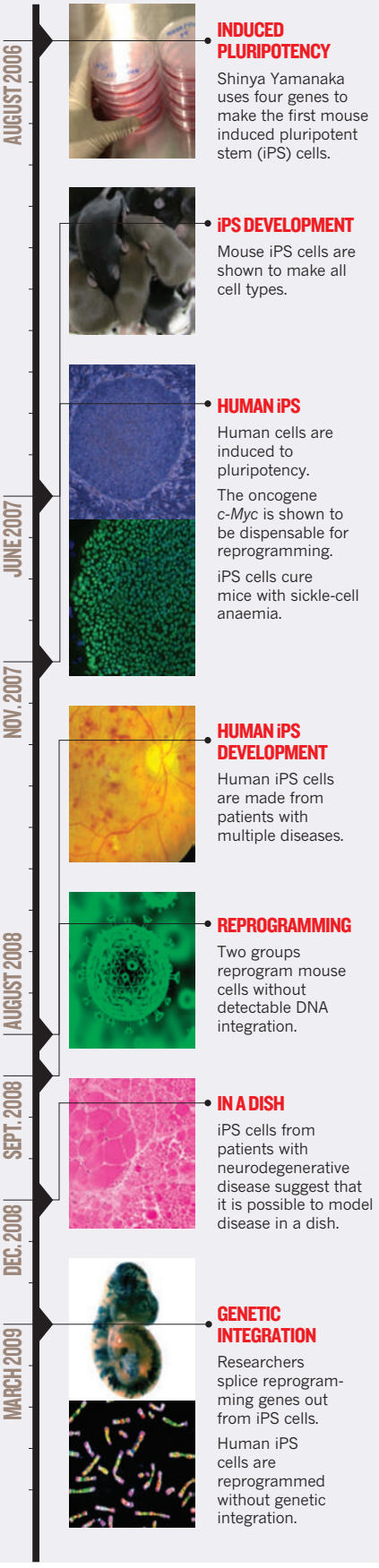
For example, a heart attack damages the heart muscle cells. Ideally, adult stem cells in the heart should be able to rebuild the muscle. For now, though, adult stem cells are limited to replacing a small number of nearby cells, not the vast damage done by a heart attack. In 2011, scientists discovered a protein that activates those adult stem cells to make new heart cells. If they can do this on a larger scale, doctors might be able to make the heart repair itself.

MEDICAL APPLICATIONS

Stem cells can teach us about the biology of pluripotent cells, how the human body develops, and what happens during disease. They are also a tool for developing safer ways to design and test new medications. Finally, they are the basis for regenerative medicine, using stem cells to repair damaged tissue.

They have enabled scientists to work out how to make their favourite differentiated cell types. In my laboratory, we study the cells that form bone and cartilage. To make these cells, we first allow colonies of stem cells — either embryonic

THE DEVELOPMENT OF iPS



or induced pluripotent stem cells — to grow in free-floating suspension cultures (as opposed to flat on a dish). This treatment causes them to start differentiating into cells of the three germ layers. Then we use growth factors to turn the cells into mesenchymal cells, an intermediate stage in the transformation to bone, cartilage and fat. Finally, we add chemicals that encourage the differentiation of bone or cartilage cells. The next step is to find out whether these cells will make bone and cartilage in an animal instead of a lab dish. We are injecting the not-quite-differentiated cells into mice to see if they continue to differentiate and form the correct structures. Other labs are exploring how stem cells can be used to repair bone and cartilage damage and heal joint and bone diseases.

Over the past decade, scientists have developed recipes to guide stem cells towards one fate or another, including cells of the heart, liver, brain and pancreas. They can then study both healthy and diseased versions of those cells. It is often easier to study individual cells than an entire person or animal. If scientists have a sample of skin from a patient they can turn it first into iPS cells and then into a cell type relevant to disease.

For example, scientists interested in spinal muscular atrophy want to study the motor neurons affected by this condition, which causes patients to lose some lower motor neurons, resulting in muscle weakness, paralysis and often death. One group, led by Allison Ebert of the University of Wisconsin–Madison, derived iPS cells and then created motor neurons from both a patient with the disease and his mother, who had no disease. The motor neurons grown from the patient's cells maintained the genetic characteristics of the disease and showed selective deficits compared with those derived from his mother[4]. Similar studies have been conducted in patients with another nervous system disorder known as familial dysautonomia.

These disease-specific cells are an important tool for drug discovery. Researchers can screen hundreds of drugs on iPS-derived motor neurons and pick the ones that seem to alleviate the pathology in the diseased cells. This kind of experiment would be unethical in humans and prohibitively expensive in animals, but it is relatively easy with cultured cells.

Researchers at Harvard University's Stem Cell Institute have made cell lines representing more than ten diseases, including type 1 diabetes, Down's syndrome and muscular dystrophy, and they plan to make many more to share with other stem-cell researchers. Another cell repository, supported by the National Institutes of Health and housed at the Coriell Institute for Medical Research in Camden, New Jersey, is also sharing iPS cells that represent diseases, including Huntington's disease and spinal muscular atrophy.

Stem cells also offer an opportunity to screen drugs for side effects before they are tested in people. For example, researchers can turn stem cells into heart cells that are differentiated enough to pulse rhythmically like the heart, even when growing in a dish. If a drug damages these cells, it is cause for concern and the drug will probably not move forward to testing in patients. Pharmaceutical companies see stem-cell-derived cultures as a way to streamline the drug testing process and make it safer. The field of drug discovery and disease models using iPS cells is moving quickly and is way ahead of regenerative medicine.

TOWARDS A TREATMENT

Doctors would like to use stem cells to treat conditions such as macular degeneration and spinal cord injuries. The few clinical trials already underway represent only a fraction of what will one day be possible using stem cells. For now, the goal is to ensure that stem-cell treatments are safe, making sure that the cells won't end up in the wrong place, cause an immune reaction or develop into cancer.

Regarding stem cell-based treatments for macular degeneration, Advanced Cell Technology is taking advantage of the eyes being a relatively safe place to start. Stem cells are less likely to be rejected in the eyes than in other organs because they are separated from the immune system by the blood–brain barrier. In addition, doctors already have all the tools they need to look into the eye and make sure the transplanted cells are behaving properly.

However, there are several steps between thinking of fixing blindness with stem cells and ultimately using them as a cure in patients. Scientists began by designing a recipe to make retinal cells from embryonic stem cells. They then tested the treatment in animals. Some rats have a version of macular degeneration, and some mice have a condition that is similar to Stargardt's macular dystrophy. When the scientists injected their retinal cells into these animals' eyes, the cells gathered near the retina and improved the animals' sight. Now the scientists are testing the safety of the treatment in a small number of people. If they can prove that the cells are safe, they will test them in more patients and find out if they actually improve vision.

"Doctors would like to use stem cells to treat conditions such as macular degeneration and spinal cord injuries."

In another exciting avenue of research, a company called Geron is running a clinical trial using nerve cells derived from embryonic stem cells to treat spinal cord injuries. The company is collaborating with researchers at the University of California, Irvine, led by Hans Keirstead. Nerves rarely grow back after injury because they lose their protective coating of myelin protein, which insulates nerves, much like the rubber insulation on an electrical wire.

Cells called oligodendrocytes are responsible for making myelin and protecting nerves from infection. The scientists reasoned that if they could replace these supportive cells they could restore the myelin around the nerves and release growth factors to repair them. First, of course, they had to work out the recipe to coax an embryonic stem cell into becoming an oligodendrocyte. After much trial and error, Keirstead's team succeeded. When they transplanted the freshly made oligodendrocytes into rats after a spinal cord injury, new myelin formed around the injured nerves and the rats regained some movement. Geron is now testing the safety of the cells in patients.

To reduce the likelihood of rejection during tissue transplant, researchers are considering using iPS cells as patient-specific transplantable material. Scientists could theoretically correct the genetic defect in iPS cells from a patient and then transplant them back into the same person. For example, Rudolph Jaenisch's research team at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, corrected the genes of mouse iPS cells to treat mice with sickle-cell anaemia. The work provided proof of principle that someday iPS cells from someone with a disease could be corrected and then transplanted back into the same person.

THE TIP OF THE ICEBERG

The discoveries and vast implications of the research described above are only the beginning. Innovative researchers around the world are exploring the impressive capabilities of stem cells by asking a range of questions.

First, what happens to transplanted stem cells? Do they go to the correct place and perform the correct functions? Second, can we ensure that stem-cell treatments do not cause cancer or other ailments? Researchers are making progress in their efforts to refine stem-cell recipes to reduce the need for cancer-causing oncogenes or viruses. Ensuring safety is essential before stem cells are used in large numbers of patients. Third, can iPS cells be used instead of embryonic stem cells, or do they retain too much 'memory' of their former lives? Fourth, will the immune system reject stem-cell transplants? And what are adult stem cells, and where do they come from? How can we get a better supply of them, and can we use drugs to make them work better?

These questions are only a small subset of those being explored in research laboratories. And there are bound to be exciting questions to pursue that we just haven't thought of yet. This growing field of research with a variety of applications in medicine has captured the attention of our culture with its potential to offer hope to those with incurable injury and disease. Clearly, stem-cell research has changed our perspective on what is possible. ■

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