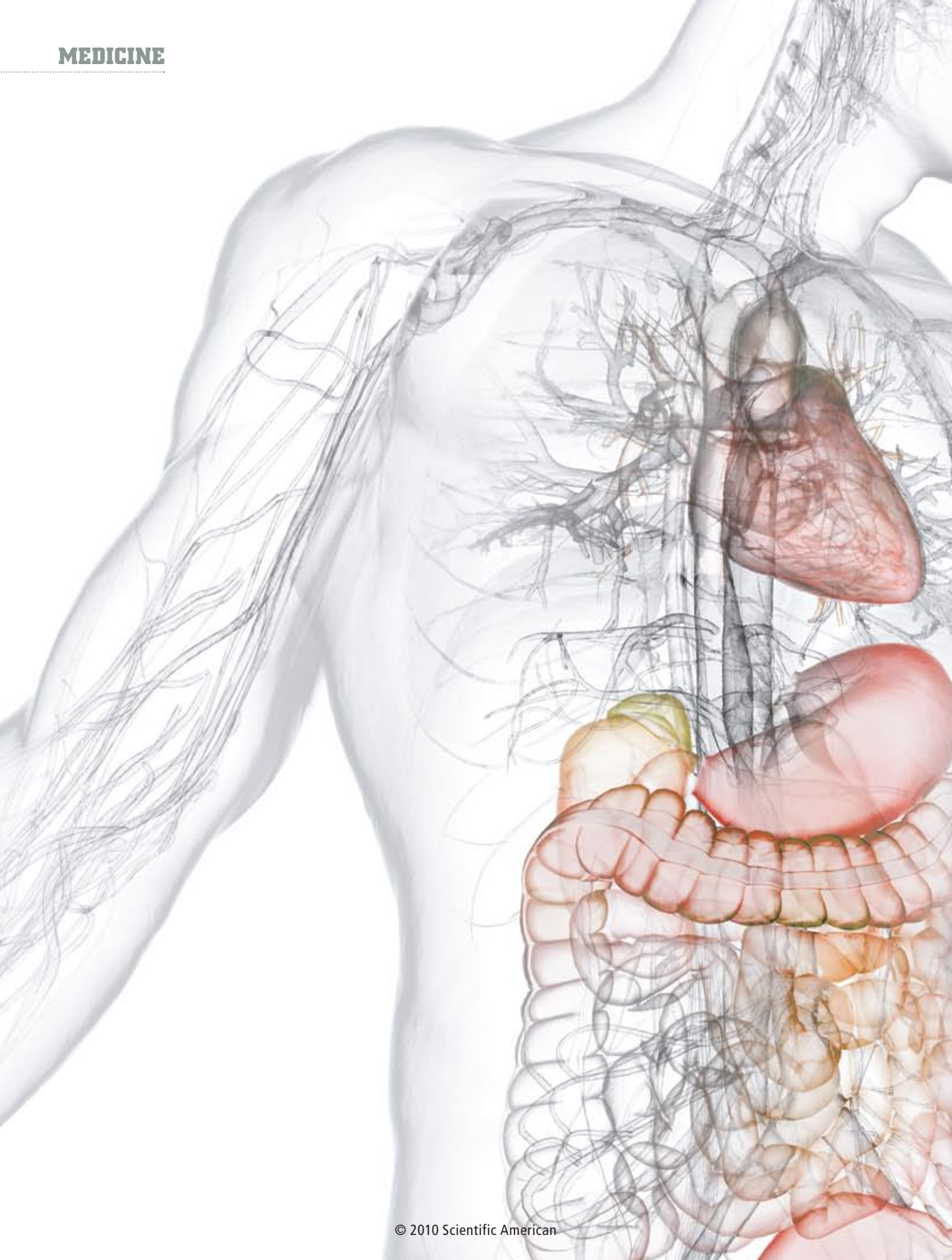
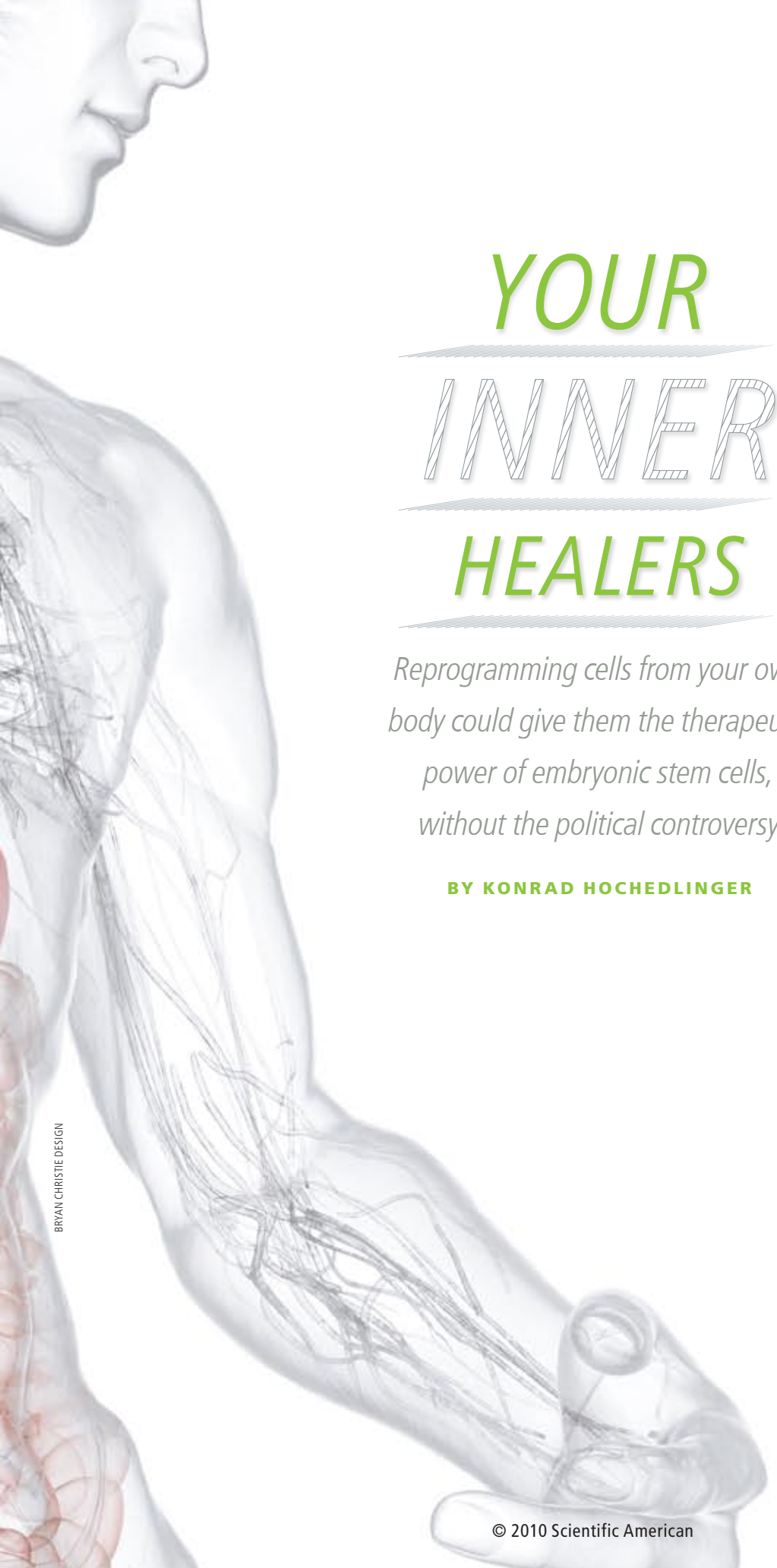




BRYAN CHRISTIE DESIGN

YOUR INNER HEALERS

Reprogramming cells from your own body could give them the therapeutic power of embryonic stem cells, without the political controversy

BY KONRAD HOCHEDLINGER

KEY CONCEPTS

- Induced pluripotent stem cells are mature body cells that have been made to change their identities and revert to an embryolike state—without the help of eggs or embryos.
- Rejuvenating the normal body cells of any individual—then converting them to any of the 220 human cell types—could yield new disease treatments and custom replacement tissues.
- Scientists are now working to understand how these cells are able to reverse their biological clocks and whether the newest kind of stem cell will prove as powerful as embryonic cells.

—The Editors

I remember my excitement one morning in the winter of 2006 when I peered through a microscope in my laboratory and saw a colony of cells that looked just like embryonic stem cells. They were clustered in a little heap, after dividing in a petri dish for almost three weeks. And they were glowing with the same colorful fluorescent markers scientists take as one sign of an embryonic cell's "pluripotency"—its ability to give rise to any type of tissue in an organism's body. But the cells I was looking at did not come from any embryo: they were regular adult mouse cells that had seemingly been rejuvenated by the addition of a simple cocktail of genes.

Could it really be so easy to roll back the internal clock of any mammalian cell and return it to an embryonic state? I was not the only one wondering at the time. Shinya Yamanaka of the University of Kyoto and his colleagues had just published a groundbreaking study in August 2006 that revealed their formula for creating what they called induced pluripotent stem cells (iPSCs) from the skin cells of mice. Researchers had been struggling for years to understand and control the enormous potential of embryonic stem cells to produce customized tissues for use in medicine and research—as well as contending with political and ethical controversies over the use of embryos, scientific setbacks and false hopes generated by previous "breakthroughs" that did not pan out. So stem cell scientists were surprised and a little bit skeptical of the Japanese group's results at first. But that morning in the lab, I could see firsthand the results of following Yamanaka's recipe.

Other scientists were also able to reproduce his achievement, and improved techniques for making and testing iPSCs have come rapidly over the past few years. Today thousands of scientists worldwide are working to develop the potential of iPSCs to help in understanding and treating human diseases that have so far defied cures, such as type 1 diabetes, Alzheimer's disease and Parkinson's disease. The possibility of changing a cell's identity just by delivering a few select genes has transformed the way scientists think about human development as well.

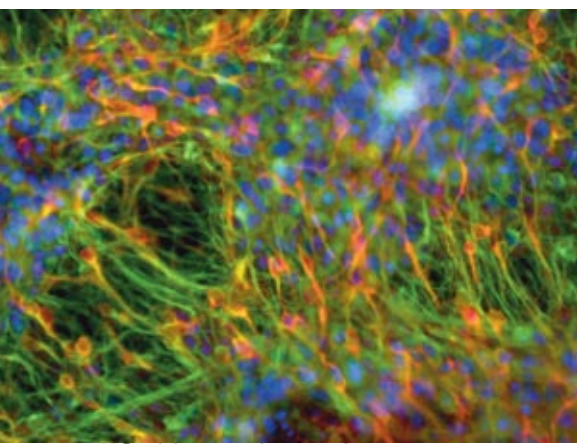
Throughout human history people have dreamed of finding a Fountain of Youth to escape the consequences of aging and disease.

Throughout history people have dreamed of finding a Fountain of Youth to escape the consequences of aging and disease, and the ability to return an adult body cell to an embryonic state would certainly appear to be as close as humanity has come to that fantasy so far. Of course, the technology is still in its infancy. Many important questions must be answered before anyone can say whether iPSCs will change the practice of medicine or even whether they will actually prove equivalent to the more controversial embryonic stem cells.

Primordial Power

To understand the hopes inspired by the discovery of iPSCs, one must return to what makes embryos so special. Current iPSC studies rely heavily on techniques and concepts developed in work with embryonic cells over the past 30 years, particularly the phenomenon of pluripotency. Mammalian development is normally a one way-street, where cells become progressively more specialized and less versatile with time, a process called differentiation. Only during a brief window very early in development do all the cells within an embryo possess the ability to become any of the 220 cell types in the human body. Extracting those cells and growing them in culture gives rise to embryonic stem cells. The ability of true embryonic stem cells to indefinitely maintain their capacity to generate any tissue type defines the term "pluripotent."

Even in a late-stage embryo, stem cells have specialized to the extent that they can give rise only to specific families of cell types, such as those in muscle and bone. These cells are considered "multipotent," but they are no longer pluripotent. In an adult, all that remains of those precursors are so-called adult stem cells that replenish mature cells within a tissue. Blood stem cells continuously regenerate the 12 different blood and immune cell types, for exam-



COURTESY OF HYESOO KIM AND LORENZ STUDER *Sloan-Kettering Institute*

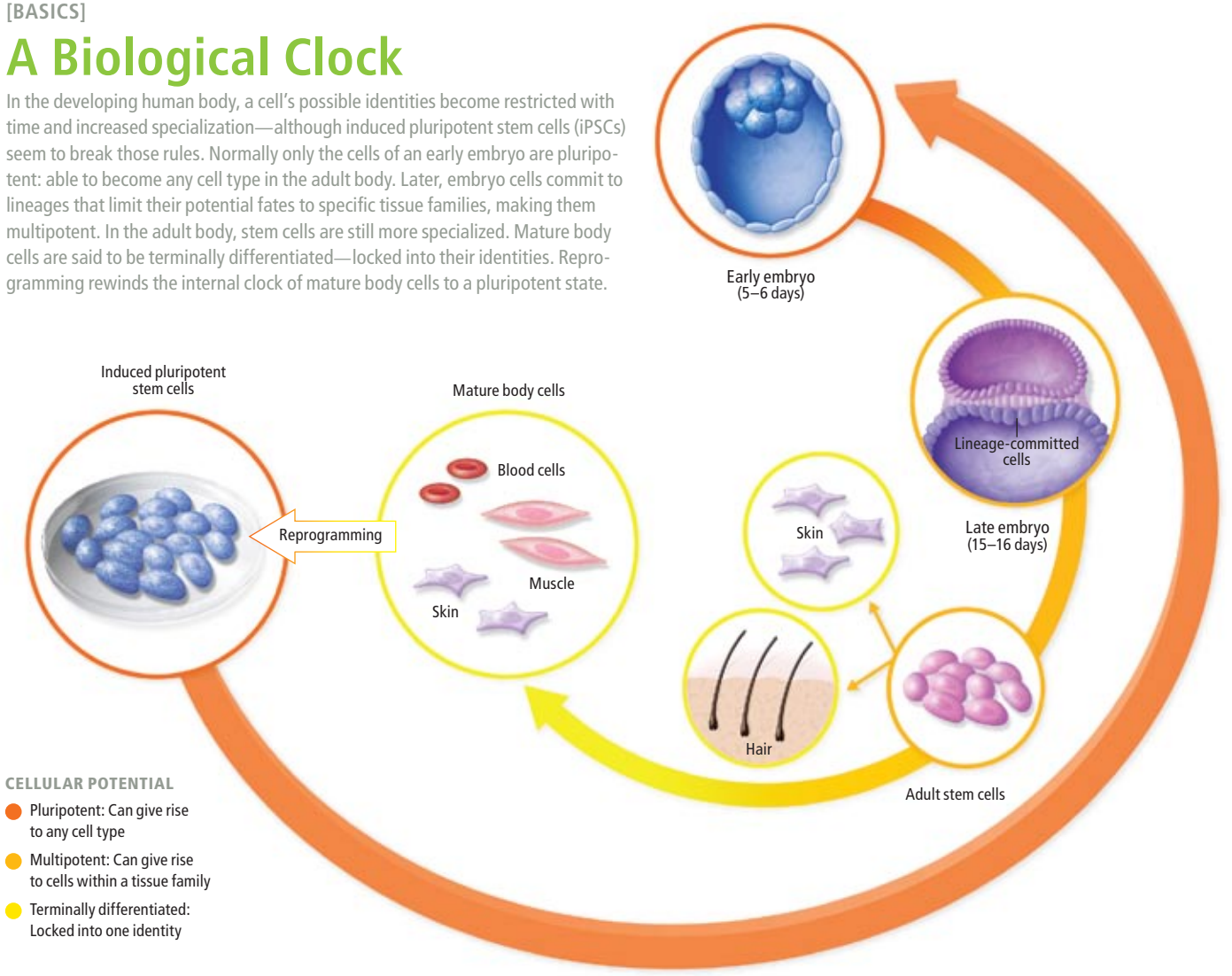
THERAPEUTIC PROMISE

Neurons (*left*) were generated from induced pluripotent cells that were made from the skin cells of patients with Parkinson's disease. With the ability to take a mature body cell and convert it to an embryonic state, then into any desired tissue type, scientists will be able to study how a variety of diseases arise, develop and test drugs that hinder the disease process and, eventually, produce healthy replacement tissues for use in treating illnesses.

[BASICS]

A Biological Clock

In the developing human body, a cell's possible identities become restricted with time and increased specialization—although induced pluripotent stem cells (iPSCs) seem to break those rules. Normally only the cells of an early embryo are pluripotent: able to become any cell type in the adult body. Later, embryo cells commit to lineages that limit their potential fates to specific tissue families, making them multipotent. In the adult body, stem cells are still more specialized. Mature body cells are said to be terminally differentiated—locked into their identities. Reprogramming rewinds the internal clock of mature body cells to a pluripotent state.



ple, and skin stem cells are responsible for regrowing our skin and hair every few weeks.

In mammals the one thing that never happens under normal circumstances is for a cell to dedifferentiate, that is, revert back to a more primitive type. Indeed, the only exception to this rule is cancer cells, which can become less differentiated than the tissue in which they first arise. Unfortunately, some cancer cells can also continue to divide endlessly, displaying an immortality similar to that of pluripotent cells.

Until recently, the only way to turn back the developmental clock of a normal adult cell was through elaborate manipulations to trick it into behaving like an embryonic cell, a process termed cellular reprogramming. The oldest approach to achieving reprogramming is somatic cell nuclear transfer, or "cloning," which involves injecting the genetic material from an adult cell into an egg cell whose own DNA has been removed. This DNA-egg hybrid then develops into an early-stage embryo from which pluripotent stem cells can be extracted.

CLONING

Transferring the nucleus of a mature cell into an egg is another method of reprogramming a person's adult DNA to an embryonic state. Attempts to derive embryonic stem cells from human-clone embryos have so far failed for unknown reasons.

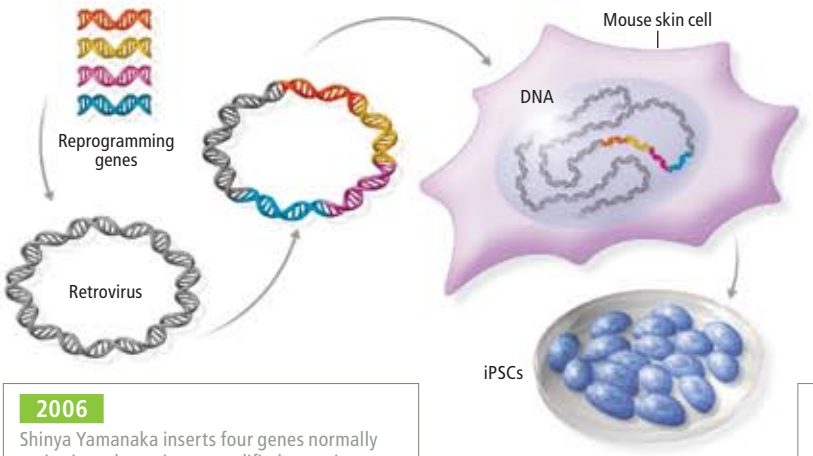
Since the cloning of Dolly the sheep was revealed in 1997 and the first isolation of human embryonic stem cells in 1998, nuclear transfer has received considerable attention as a possible means of producing custom-tailored pluripotent stem cells to replace any tissue damaged through injury or disease. Poorly understood factors within the egg do seem to genuinely rejuvenate the genetic material of the adult donor cell—even telomeres, the caps protecting the ends of chromosomes that wear away with age, are restored to a youthful state. Yet despite progress with animals, attempts to produce human embryonic stem cells through cloning have remained unsuccessful.

Yamanaka and his group went around this impasse by taking a novel approach to turning

TAMI TOLPA (*illustration*); PHILIPPE PSAILA *Photo Researchers, Inc.* (*stem cell nuclear transfer*)

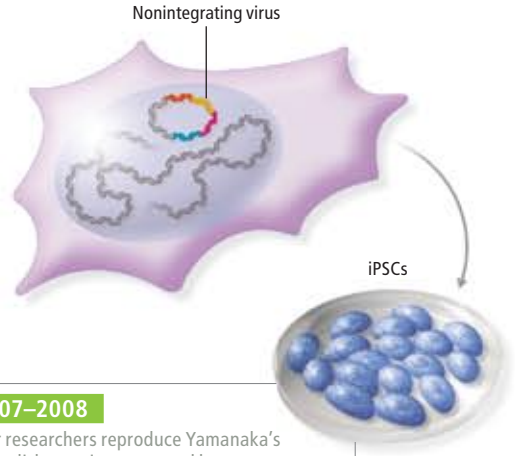
Rapid Progress toward Safe Cell Rejuvenation

Just four years ago scientists in Japan first showed that a set of genes ferried by a retrovirus could transform the skin cells of adult mice into pluripotent stem cells. Many researchers have since been working to achieve the same end in simpler, safer and more efficient ways—key steps to making therapy a reality.



2006

Shinya Yamanaka inserts four genes normally active in embryos into a modified retrovirus, which he then injects into mouse skin cells. The virus inserts the genes into the mouse DNA, and the genes then begin reprogramming the skin cells into induced pluripotent stem cells (iPSCs).

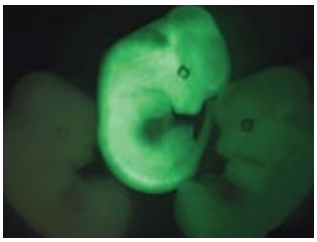


2007–2008

Other researchers reproduce Yamanaka's accomplishment in mouse and human cells. Experiments also show that delivery of the four reprogramming genes by viruses that do not permanently integrate into cellular DNA still succeeds in producing iPSCs.

adult cells directly into pluripotent cells without the use of eggs or embryos. Instead of introducing adult genetic material into an egg, they reasoned that introducing the genes normally active only in embryos into an adult cell might be sufficient to reprogram that cell into an embryolike state. Their first feat was to identify a cocktail of two dozen different genes that are turned on in pluripotent cells but silent in adult cells. When introduced into skin cells using retroviruses as delivery vehicles, these genes then almost magically reprogrammed the identity of the skin cells into that of pluripotent cells. With further experiments, Yamanaka then found that only four genes—*Oct4, Sox2, Klf4* and *c-Myc*—were actually necessary to produce iPSCs.

As soon as several independent laboratories, including mine, successfully reproduced the results, this magic trick became a biological fact. By now about a dozen different adult cell types from a total of four different species (mouse, human, rat and monkey) have been reprogrammed into iPSCs, and certainly more will follow. The discovery of iPSCs is so thrilling to stem cell researchers because they can circumvent the technical complexities of cloning and avoid most of the ethical and legal constraints associated with human embryo research. This new pluripotent cell type is not without its own problems, however. Quality control and safety are the main focus of iPSC research right now, as scientists work to establish what these cells really are and what they are capable of doing.



TESTING CELLS' TRUE POTENTIAL

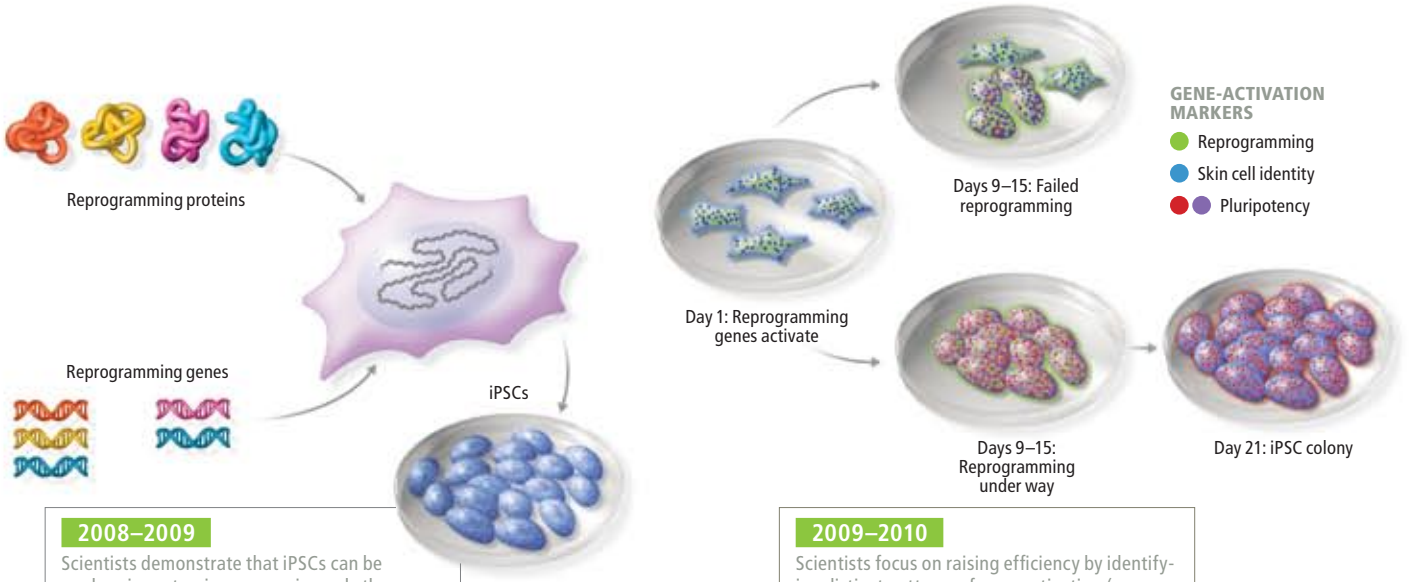
Gold-standard laboratory tests to determine whether stem cells are truly pluripotent aim to demonstrate that the cells can give rise to any tissue type in the body. When injected into an early mouse embryo, for example, fluorescently marked pluripotent cells should integrate throughout the body of the developing mouse (*bright green, above*). Finding alternative methods of verifying the pluripotency of human iPSCs is an important goal.

Identity Crisis

Although iPSC colonies may look like embryonic stem cells under a microscope and may display the molecular markers associated with pluripotent cells, the unequivocal proof of their pluripotency comes from functional testing—can the cells do all the things a pluripotent cell, by definition, can do? Even embryo cell colonies can contain some dud cells that do not display the pluripotency of a true embryonic stem cell, and scientists have developed a few routine tests to gauge a cell's pluripotency. With increasing stringency, they are: the ability of stem cells to produce a wide variety of body cell types in a petri dish when exposed to the appropriate developmental cues; the ability of stem cells to produce a teratoma (a type of tumor containing cells from all embryonic tissue lineages) when injected under the skin of a mouse; and the capacity, when injected into an early-stage mouse embryo, to contribute to the development of all tissue lineages, including germ cells, in the resulting newborn mouse.

Whereas embryonic stem cells generally pass all these tests, many iPSCs do not. Closer examination of the cells that fail has revealed that the viruses used to deliver the four key repro-

TAMI TOLPA (*illustration*); COURTESY OF ALEXANDER MEISSNER *Harvard University* AND MARIUS WERNIG *Stanford University* (*mouse embryos*)



gramming genes into skin cells are often not properly shut off, and important genes in the cells' original DNA are not properly turned on, resulting in cells that have lost their skin cell identity without gaining a pluripotent identity. These partially reprogrammed cells therefore do not qualify as authentic pluripotent cells.

Ongoing studies of iPSCs that do pass all the pluripotency tests are aimed at pinpointing the differences that distinguish a "good" from a "bad" iPSC. Thorsten Schlaeger, George Daley and their colleagues at Harvard University, for example, recently identified a pattern of gene activity in skin cells undergoing the lengthy (about three weeks) process of changing their identity to that of pluripotent cells. The fluorescent markers displayed by these cells during the transition distinguished them from cells in the same colony that would not ultimately become iPSCs, and so this marker pattern could be used as an early indicator of successful conversion.

Because scientists cannot ethically perform the most stringent pluripotency test by injecting human iPSCs into human embryos, it is absolutely critical to ensure that human iPSCs fulfill all other criteria of pluripotency. These include the complete silencing of the potentially harmful viruses employed to deliver the reprogramming genes. Yamanaka's team members discovered, for example, that one third of the mice that they had generated by injecting iPSCs into developing mouse embryos later formed cancers as a consequence of residual retrovirus activity.

One of the main problems with using retroviruses as gene-delivery vehicles is that these kinds of viruses (HIV is one example) integrate themselves directly into the host cell's DNA strand, becoming a part of its genome. This ability allows the added genes to reside permanently and remain active in the host cell, but depending on where the virus inserts itself, it can cause DNA damage that sparks cancerous changes in the cell. In efforts to produce safer iPSCs, therefore, many labs have developed methods that avoid permanent genetic manipulation of cells.

My research group has used a modified type of adenovirus, which normally causes the common cold in humans, to deliver the four reprogramming genes into mouse cells without integrating into the cellular genome. Adenoviruses persist inside the cells for only a short period—just long enough to convert them into iPSCs. When we injected the resulting pluripotent cells into mouse embryos, they readily became incorporated into the developing animals, which were all tumor-free as adults. This discovery, along with several alternative approaches to producing virus-free iPSCs, should eliminate a major roadblock to one day applying iPSCs directly in human therapies.

[THE AUTHOR]

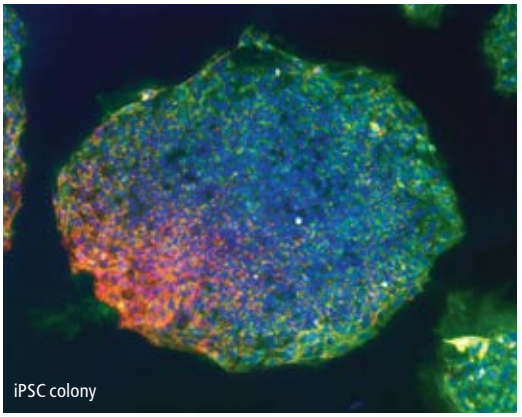
Konrad Hochedlinger is associate professor of stem cell and regenerative biology at Harvard University and a faculty member of the Harvard Stem Cell Institute and the Howard Hughes Medical Institute. In his laboratory at Massachusetts General Hospital, he works toward understanding the biology of stem cells and cellular reprogramming and their potential use in the treatment of disease. He is also a scientific adviser to iPierian, a biopharmaceutical company developing products based on stem cells.

COURTESY OF SAM RILEY *Massachusetts General Hospital*

[THERAPY POSSIBILITIES]

Custom-Tailored Cells to Cure Disease

An ability to transform a patient's skin or blood cells into iPSCs and then into any other type of cell could cure diseases in two ways: in the very near future, by allowing scientists to "model" illnesses and test drugs in a petri dish and, perhaps in another decade, by repairing or replacing diseased tissues.



iPSC colony

APPLICATION	STATUS
DISEASE MODELING Convert iPSCs derived from patients into the affected tissue type, then study disease progression and drug responses in those cells	■ Human iPSCs have already been used to generate 12 tissue types, including cells representing diverse disorders such as Parkinson's disease and diabetes ■ Symptoms of smooth muscular atrophy and familial dysautonomia have been "treated" in cultured cells
CELL THERAPY Convert iPSCs derived from a sick patient into healthy cells for transplantation into that individual	■ 10 years or more in the future ■ iPSC-derived neurons have been transplanted into rats to treat a version of Parkinson's ■ iPSC-derived blood progenitor cells with corrected sickle cell anemia genes cured the disease in mice

Ultimately, researchers hope to produce iPSCs without using any type of virus, but instead by simply exposing adult cells to a combination of drugs that mimic the effect of the reprogramming genes. Sheng Ding of the Scripps Research Institute, Douglas A. Melton of Harvard and others have already identified chemicals that can substitute for each of the four reprogramming genes in that each chemical activates a pathway of molecular interactions inside a cell that would be activated by the gene. When the four drugs have been tried together, however, they proved insufficient to make pluripotent cells. It may only be a matter of time, though, until researchers find the right cocktail and concentration of drugs to reprogram body cells into iPSCs without ever using viruses.

Healing Cells?

Because pluripotent cells are capable of generating any type of tissue in the body, the application that most captures the public imagination is the possibility of using iPSCs to produce replacement parts for cells and organs damaged by disease: neurons lost to Parkinson's or a spinal cord injury, for instance, or cardiac tissue destroyed by a heart attack. The ability to convert adult cells from the intended recipient of such a transplant into pluripotent cells and then coax those cells into the desired tissue would mean the replacement part is perfectly matched, genetically and immunologically, with the recipient's body. Moreover, easily accessible skin cells could be used to produce any kind of needed cell, including those in hard-to-reach organs and tissues, such as the brain or pancreas.

ETHICS UNCLEAR

Injecting iPSCs into a developing mouse embryo yields a chimeric animal (*above*) that displays the presence of foreign cells in its mixed coat colors. The same technique could, in theory, create a chimeric human embryo; iPSCs could also theoretically generate sperm and eggs to produce a human embryo through traditional in vitro fertilization. The pluripotency of iPSCs thus could raise some of the same ethical issues as human embryo research.

This technique also offers the possibility of repairing disease-causing genetic mutations before reintroducing the new cells, an approach that has been used with the adult stem cells that naturally regenerate some tissues. Success has been limited, though, because those precursor cells are notoriously difficult to grow and manipulate outside the body.

Recent experiments in mice suggest that treating genetic disorders in this manner with iPSCs is indeed feasible. Specifically, Rudolf Jaenisch of the Massachusetts Institute of Technology showed in 2007 that iPSCs could cure sickle cell anemia in an animal. The disease results from a single genetic mutation that causes red blood cells to adopt a deformed crescentlike shape. In this proof-of-concept study, investigators first reprogrammed skin cells from the mice into iPSCs. They then replaced the disease-causing gene in the iPSCs with a healthy version and coaxed the "repaired" iPSCs into becoming blood-forming stem cells. After transplantation back into the anemic mice, the healthy precursors produced normal red blood cells. In principle, this method could be applied to any other disease in humans for which the underlying gene mutation is known.

The multimillion-dollar question is how long it might take before iPSCs can be used to treat people. For the reasons already outlined, safety and control are absolutely essential before any iPSC-derived cells could be tested in humans. Current strategies to push embryonic stem cells or iPSCs into fully differentiated mature cell types cannot yet efficiently eliminate the occasional immature stem cells that might

COURTESY OF WILLIAM COLLINS *Deepak Srivastava Lab, Gladstone Institute of Cardiovascular Disease (iPSC);* COURTESY OF KONRAD HOCHEDLINGER AND MATTHIAS STADTFELD *Massachusetts General Hospital (mouse)*

seed a tumor. An example underscoring why this is such a problem comes from a recent experiment in transplanting iPSC-derived dopamine-making neurons, which are the cells lost in Parkinson's patients, into rats suffering a version of the human disease. Although the rats clearly benefited from the engrafted cells, some of the animals also eventually developed teratomas in their brain.

In light of the fast pace of discoveries so far, however, it is optimistic but not unreasonable to estimate that such obstacles could be overcome in as little as 10 years, and transplantation of iPSC-derived cells might then be ready for human testing to begin. But iPSCs could well demonstrate their therapeutic value much sooner. The study and treatment of many tissue-destroying diseases, such as type 1 diabetes, Alzheimer's and Parkinson's, are limited by scientists' ability to obtain the affected tissues for study or to grow them in cultures for extended periods, and iPSCs could therefore be of enormous service in so-called disease modeling.

The idea is to derive iPSCs from affected patients' skin or blood cells and then convert them into the cell types involved in the patients' diseases. Both Clive N. Svendsen of the University of Wisconsin–Madison and Lorenz Studer of the Sloan-Kettering Institute recently derived iPSCs from the cells of patients with the devastating disorders smooth muscular atrophy and familial dysautonomia, respectively. When the iPSCs were transformed into the cell types affected in each of those diseases, the cultured cells recapitulated the abnormalities just as they are seen in patients.

This process could allow researchers to study the development of a disease in a petri dish, with the advantage of having a potentially endless supply of new cells, because the original iPSCs can be maintained indefinitely. Ultimately, the goal of academic scientists as well as pharmaceutical companies is to use these petri dish models to better understand the disease process and identify novel drugs to treat the illness.

This extremely promising use of iPSCs is not far off at all. Indeed, when Svendsen and Studer exposed their cell cultures to experimental drugs in each study, the disease "symptoms" were partially alleviated in the cells. This principle can now be applied to many other disorders for which treatments do not yet exist, and unlike transplanting cells into individuals, the result may be the development of drugs from which millions could benefit.

CELLS FOR SALE

The first commercially marketed product made from human iPSCs, a heart cell line called iCell Cardiomyocytes, is intended for use by pharmaceutical companies to test the effects of potential heart drugs.

MORE TO EXPLORE

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Kazutoshi Takahasi and Shinya Yamanaka in *Cell,* Vol. 126, No. 4, pages 663–676. Published online August 10, 2006.

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Induced Pluripotent Stem Cells and Reprogramming: Seeing the Science through the Hype. Juan Carlos Izpisúa Belmonte, James Ellis, Konrad Hochedlinger and Shinya Yamanaka in *Nature Reviews Genetics,* Vol. 10, No. 12, pages 878–883. Published online October 27, 2009.

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Challenges and Hope

Although iPSCs clearly circumvent some of the ethical and legal controversies surrounding embryonic cells, their pluripotency has yet to be completely understood or controlled, and embryonic stem cells therefore remain the gold standard for any pluripotent cell type.

Important unanswered questions include the practical issue of whether the conversion of body cells into iPSCs and the conversion of iPSCs into therapeutically relevant cell types can ever be made efficient enough for widespread use. Also unresolved is whether iPSCs retain any memory of the body cell type from which they are derived, a factor that could limit their ability to be converted into any other type of cell. We have gained some insight into the mechanisms by which a mature cell transforms into a pluripotent cell, but the process of reprogramming—how only a few genes manage to rewire the entire program of a mature cell into that of an embryonic cell—is still largely a black box.

Tackling such questions will require the continued use of embryonic cells as a reference point and will determine whether embryonic stem cells may be more effective for certain types of applications and iPSCs for others. Moreover, as truly pluripotent cells, iPSCs may raise ethical issues similar to concerns over embryonic cells because, in theory at least, iPSCs could be used to generate human embryos [*see box on opposite page*].

Nevertheless, from a scientific standpoint progress in the field of cellular reprogramming in recent years is truly astounding. Advances in cloning and, more recently, the discovery of iPSCs have refuted the old dogma that the identity of cells is irreversibly locked once they have differentiated. Both techniques have raised the possibility, at least, of reprogramming the identity of a body cell from one type of tissue into that of any other tissue type just by manipulating a few genetic switches. Understanding how this rewiring works at a mechanistic level will keep researchers energized and busy for years to come.

Only time can reveal whether iPSCs or related technologies will indeed become the modern Fountain of Youth. I personally think there is a good chance they will. Certainly iPSCs will continue to influence approaches to the study and treatment of many devastating diseases and have the potential to revolutionize medicine in the 21st century as profoundly as vaccines and antibiotics did in the 20th century. ■