





## Sources of Renewal

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As scientists learn more about how to produce and manipulate stem cells—amid high expectations and close scrutiny—no one is ready to choose any one approach over another.

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by **Robin Meja**  
illustration by Shout



Above the desk of HHMI investigator  
George O. Daley hangs a striking  
impressionistic painting of a person  
lost in thought, called  
“On Learning My Diagnosis.”

It's by an attorney Daley began treating in 1993 for a chronic and sometimes fatal blood disorder. With that diagnosis, the man put down his law books. He has been doing so well for so long, however, that he's returned to his legal practice. This is the kind of outcome that a doctor hopes for.

Not every patient is that lucky. “I just spent the last week in the hospital taking care of kids with all kinds of blood diseases,” says Daley, a physician-researcher at Children's Hospital Boston. “Sickle cell anemia, for example, is exquisitely painful, and we don't have a treatment for it. We give them narcotics and we hydrate them, but what we'd ideally like to do is repair their cells.”

He envisions a day when he'll be able to take cells from his patients, repair the damaged genes, and grow new blood cells to treat them. Daley, a leader in studying stem cells—embryonic stem (ES) cells, adult stem cells, and the tantalizing but still very new induced pluripotent stem (iPS) cells—has already managed to do that kind of cellular repair in mice.

For Daley and others who study stem cells, recent discoveries make this an exciting and challenging time. They are learning how to manipulate these cells to produce the building blocks of various organs. And the development of iPS cells opens the possibility of producing patient-specific stem cells without using human embryos, an achievement that could defuse many of the ethical and political tensions that surround this area of biology. But there is much work to do before researchers know how well these stem cells will deliver on their promise.

### Working within Limits

*To achieve cellular repair in mice, a team led by senior colleague Rudolf Jaenisch and Daley, when he was at the Whitehead Institute, took the nucleus from the cell of an adult mouse with a genetic defect and inserted it into a mouse egg. (An egg cell can*

reprogram its nucleus to create a cell with the potential to produce any part of the body.) If the egg is grown to a blastocyst—an early stage embryo—researchers can harvest stem cells from it. Daley's team did that and more. They repaired the defective gene in the cultured stem cells, coaxed the repaired cells to become blood stem cells in a Petri dish, transplanted the healthy blood cells into diseased mice, and partially restored immune function in the animals; they published the results in 2002 in *Cell*.

This is the ultimate promise of stem cell research—fixing illnesses at the genetic level and then using the modified cells to treat patients. No one has yet succeeded in creating human stem cell lines with the technique Daley's team used in mice, called somatic cell nuclear transfer or therapeutic cloning. Researchers have created human stem cell lines from embryos donated by in vitro fertilization patients, but these cell lines are not patient specific.

There are groups with ethical or religious concerns that consider the use of embryos destruction of human life or a step toward reproductive cloning. President George W. Bush agrees, and announced in 2001 that the U.S. government would fund research only with human embryonic stem cell lines created before August 9, 2001. Researchers like Daley cannot apply for National Institutes of Health (NIH) grants, or any other federal funds, to support development of new human embryonic stem cells, nor can they use equipment funded by federal grants to work with newer stem cell lines.

Voters in some states as well as private donors have provided a few alternatives. In 2004, Californians passed Proposition 71, authorizing \$3 billion in state bonds to fund stem cell research through a granting agency called the California Institute for Regenerative Medicine. A handful of other states followed, with much smaller amounts. For its part, Harvard relied on private philanthropic donations to create the Harvard Stem Cell Institute, which now encompasses labs at the medical school, other parts of the university, and 11 teaching hospitals. Daley, a member of the Stem Cell Institute along with several HHMI colleagues, lined up private funding to support his embryonic stem cell work, supplemented in February 2008 when he became an HHMI investigator.

However, the administration's position on human embryonic stem cells is seen as a barrier, as many researchers limit themselves to projects that are eligible for funding from the NIH.

“I have a junior investigator in my lab who's a driving force behind our human ES research, which uses all private funding,”

says Daley. “As he’s trying to get an independent faculty position, other mentors are saying, you need an NIH grant, you better focus on a mouse program.”

### Excitement Tempered with Caution

*At the end of 2007, a new door swung open. Scientists in Japan and Wisconsin, and Daley at Harvard, reported that they had successfully turned human adult skin cells into stem cells. In November, researchers in the lab of Shinya Yamanaka at Kyoto University documented in *Cell*, and, separately, a team led by James Thomson at the University of Wisconsin reported in *Science*, that they had created stem cells by inserting four genes into human adult skin cells. The genes appeared to perform the same function that insertion into an egg does in other animals: resetting the cell’s genetic state back to day one.*

Daley’s paper, published online in *Nature* in December 2007, described his ability to reprogram human adult skin cells using even fewer genes. Scientists refer to the new cells as induced pluripotent stem (iPS) cells, meaning they have been coaxed to regress to a state in which they could become any of the various cell types that make up the body. In human cell lines, such pluripotency is shared only by embryonic stem cells, although the iPS technique bypasses one of the steps that has hampered the development of patient-specific human lines; the new process created stem cells without the costly and difficult step of harvesting eggs. And no embryos are required.

Researchers around the world are elated by this apparent breakthrough. “Clearly, this work is a very big step,” says Alan Trounson, president of the California Institute for Regenerative Medicine, which was founded to support embryonic stem cell research but now expects to fund efforts using the new technique as well.

Some researchers are even transcending their usual penchant for understatement. In *The New England Journal of Medicine*, Douglas R. Higgs, of Oxford’s Weatherall Institute of Molecular Medicine, pointed out iPS cells’ clinical implications, particularly their potential for overcoming the immune system incompatibility issues of existing transplant technology. Reprogramming a patient’s own somatic cells, he wrote, is “the biologic equivalent of an alchemist’s dream of turning lead into gold.”

But iPS cells are a new discovery with plenty of questions that need exploring. Daley notes that while iPS work holds promise as

## TWO STEPS CLOSER

In type 1 diabetes, the body attacks and kills its own insulin-producing pancreatic beta cells, leaving patients dependent on insulin injections for life. Unlike the liver and skin, the pancreas does not have a well of adult stem cells at the ready to repair damage. When the supply of beta cells is exhausted, as happens in type 1 diabetes, there’s no place to turn for more.

HHMI investigator Douglas A. Melton’s sights are set on curing type 1 diabetes by regrowing beta cells, and he is trying to do so the only way he can, from embryonic stem cells. Guiding the ES cells through several developmental steps is a long, slow process.

“Figuring out how to tell these cells, which we know can do anything, what to do is a challenge,” he says. Researchers can tease stem cells into some types of neurons and blood cells, but Melton estimates that five to seven steps are required to create beta cells—and it has taken three years to figure out how to guide ES cells through just two of those steps.

This is stem cell research as developmental biology. Melton explains that the most immediate benefits of most stem cell research will likely come from what these cells can teach scientists about how cells differentiate, how tissues develop, and how disease occurs.

For example, once Melton discovers how to grow a beta cell, he could create stem cells from someone with type 1 diabetes and from someone else with healthy beta cells. Then he could grow both stem cells into beta cells and watch to see where the diabetic’s cells go wrong.

By producing the disease in a Petri dish, scientists can run experiments—and even test drugs—in ways they never could in people. This is why the Harvard Stem Cell Institute has spent more than \$6.5 million creating a lab to facilitate precisely that kind of work.

Thus, in the basement of the building where Melton conducts his research, a collection of boxy robots is at work. They hold plates barely larger than drink coasters, with each containing up to 384 cultures in separate tiny wells. Mechanical limbs move the plates around and drop different chemicals into each—creating, in effect, 384 distinct experiments. Next door, an automated microscope reader scans the results of these experiments; it takes up to two hours to scan all 384 wells on a plate.

That’s still a lot faster than a postdoc. “In fact, a person couldn’t really do that assay,” says Lee Rubin, director of translational medicine at the Harvard Stem Cell Institute. This is why most academic labs have traditionally studied only a few compounds at a time. Harvard researchers are using Rubin’s robotic set-up—a stripped-down version of a drug-company screening lab—to ask a broad array of scientific questions. And it is how Melton figured out how to coax his embryonic stem cells two steps closer to a pancreas. —R.M.



From left:

**George O. Daley**, HHMI investigator  
**Douglas A. Melton**, HHMI investigator  
**Sean J. Morrison**, HHMI investigator  
**Stuart H. Orkin**, HHMI investigator  
HHMI scientists and others are exploring the possibilities and limitations of stem cells to understand tissue development with hopes of curing disease.

## PRINCIPAL PLAYERS

**Embryonic stem (ES) cells** are derived from embryos that develop from eggs fertilized in vitro. Each embryo, typically four to five days old, is a hollow microscopic ball of cells called a blastocyst. To generate cultures of specific types of differentiated cells—heart muscle cells, blood cells, or nerve cells, for example—researchers change the chemical composition of the culture medium, alter the surface of the culture dish, or modify the cells by inserting specific genes. Through years of experimentation, scientists have established some basic protocols, or “recipes,” for the directed differentiation of embryonic stem cells into specific cell types.

**Adult stem cells** are undifferentiated cells found among the differentiated cells that constitute a tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue where they are found. The adult tissues reported to contain stem cells include brain, bone marrow, skeletal muscle, skin, and liver.

**Somatic cell nuclear transfer** reprograms adult cells, a feat that has been accomplished only with animal cells. An adult cell’s nucleus is inserted into an egg cell, which creates an embryo. Stem cells are then derived through a process similar to that used to create embryonic stem cells.

**Induced pluripotent stem (iPS) cells** are derived from adult somatic cells such as skin cells. These cells are reprogrammed, with the insertion of a handful of genes, to act as stem cells. In fact, they then display embryonic stem cell-like abilities.

an easier route to his goal of making patient-specific stem cells, none of his physician colleagues would consider using iPS cells as a treatment, at least for now. To insert new genes into cells to form iPS cells, researchers attached the genes to fragments of a virus that can cause cancer.

Scientists are working on methods to revert cells to a pluripotent state without using such viruses—by employing drugs, for example, or by injecting proteins directly into the cell.

“I think we’ll see this happen soon,” says Konrad Hochedlinger, a colleague of Daley’s at the Harvard Stem Cell Institute who also works on iPS cells. “Then the big question will be how similar these induced cells are to embryonic stem cells.”

“People take it for granted that they are identical,” he says, but iPS cells are not yet as well understood as their ES counterparts. Thus, Hochedlinger wants to grow human iPS cells alongside human ES cells and then direct both to become adult tissues such as muscle or nerve cells. He points out that with mouse cells, molecular analysis of the two cell types found no major differences, but when he attempted to grow adult heart cells from the mouse iPS cells using a protocol developed with ES cells, the iPS cells didn’t seem to form tissue as easily.

“Superficially, things look okay, but as you look more closely, the iPS cells don’t develop quite normally,” says HHMI investigator Stuart H. Orkin, at Children’s Hospital Boston. “They’re pretty close but they ain’t perfect.”

Orkin studies ES cells, trying to understand exactly what processes keep them from differentiating into adult tissue. The initial iPS experiments reported last fall created something of a “black box,” he explains. Scientists know that four specific genes cause the cells to regress to a pluripotent state, but they don’t

# “We’re gathering a complete parts list of the things that are involved and required.”

Stuart Orkin

know how they do it, or why the process takes weeks longer than transferring a nucleus from an adult cell into an egg.

In a series of experiments described in the March 21, 2008, issue of *Cell*, Orkin examined nine genes that are known to help ES cells maintain themselves, including the four used in the recent iPS experiments and five others. Each of those nine genes produces a transcription factor, a protein that causes other genes to turn on or off. Orkin identified hundreds of genes that are targeted by one or several of the transcription factors, work that he hopes will help scientists tease out the cellular-level processes that help ES cells maintain and reproduce themselves. Similar work on iPS cells could help explain the differences between the two types of stem cells.

“We’re gathering a complete parts list of the things that are involved and required,” Orkin says. “Maybe adding something that hasn’t been considered yet might make it better.”

## Science Informing Policy

*With each new discovery, stem cell researchers have learned to provide perspective and context to help a hopeful public and those eager to find alternatives to using human embryos to understand the implications of the findings and the questions that remain.*

HHMI investigator Sean J. Morrison, director of the University of Michigan Center for Stem Cell Biology, testifies to state and national officials about stem cell research, writes op-eds, and frequently talks with reporters.

Douglas A. Melton, a co-founder of the Harvard Stem Cell Institute, has discussed stem cell policy with President Bush. The HHMI investigator doesn’t particularly enjoy policy work, but thinks it is important.

The iPS findings added to the ongoing debates. In his State of the Union Address on January 28, 2008, President Bush noted, “In November, we witnessed a landmark achievement when scientists discovered a way to reprogram adult skin cells to act like embryonic stem cells. This breakthrough has the potential to move us beyond the divisive debates of the past by extending the frontiers of medicine without the destruction of human life.” He urged Congress to pass a ban on cloning, which would preclude

the development of stem cell lines created by somatic cell nuclear transfer, the technique Daley used in his mouse work and continues to explore with human cells.

This ban is a long-standing goal of groups opposed to human embryonic stem cell research. They praised the iPS work and used it as a reason to call (again) for a ban on therapeutic cloning.

“These are the same political lobbyists that have been looking for reasons to end this research all along,” says Morrison, who studies the mechanisms involved in adult stem cell renewal and aging. “The positions they’ve taken in the past were not credible, and the position they’re taking on iPS cells is not credible.” He points to earlier claims by several groups that 65 diseases had been treated with adult stem cells. That claim, “has been roundly dismissed.”

“We haven’t had a logical debate,” adds Melton, who argues that if stem cell research were truly unethical, “you would never say it’s okay as long as you don’t use federal funds.”

Both Melton and Morrison are hopeful that scientists may eventually be able to focus only on iPS cells, but they argue that it’s too early to close the door on embryonic stem cell research. “Certainly from a patient perspective that would be wrongheaded,” says Melton, whose position is echoed throughout the field.

This caution is based on history. In the early 1990s, researchers thought they were closing in on treatments for genetic diseases when they figured out how to insert working copies of genes directly into patients’ cells. “We could get genes to express in bone marrow,” says Daley, but those genes were inserted into cells by using viral vectors much like those involved in creating iPS cells. Because that treatment led to an unanticipated side effect—leukemia—iPS cells are not considered safe for human transplants.

“Some people believe that these are just technical obstacles we will overcome,” says Morrison. “Others believe that the Food and Drug Administration will never approve these lines, even if we improve the technology and eliminate the viruses.” He pauses

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(GENETIC BALANCING ACT)

As her team revealed this March in the *Proceedings of the National Academy of Sciences*, two of the stem cell lines carried out X inactivation just fine. But in six lines, after one X was inactivated the cells stopped producing *Xist* RNA. Although the team found no evidence that an entire X chromosome reawakened in these cell lines, it's possible that some—perhaps many—genes on the X could fire up again. The scientists have already found evidence that this happens in mouse stem cells.

Reactivation might just kill the cells, but it could spell trouble for another reason. Some tumor cells carry an extra X chromosome, so it's not unreasonable to wonder whether a partially reactivated X might prompt similar abnormal growth. "It's extremely disconcerting," says Lee. "There's nothing we can do to restore X inactivation once reactivation occurs." The findings, she says, indicate that researchers need to do more experiments to determine

whether stem cells induce tumors if they are transplanted into patients.

Other stem cell experts praise this work. Although researchers have previously pinpointed X inactivation mishaps in stem cells, "this is the most thorough study" to date, says Renee Reijo Pera, director of the Center for Human Embryonic Stem Cell Research at Stanford University. "It definitely raises a red flag," though we need more information about X inactivation in the early embryo to judge how serious the problem is, she says.

#### Expect the Unexpected

What intrigues Meyer these days is the connection between dosage compensation and other cellular events that involve large-scale alterations to chromosomes. One example is crossing over, which occurs during meiosis, the type of cell division that leads to sperm and eggs. During crossing over, chromosomes pair up and swap DNA. The exchange is important from an evolutionary standpoint because it boosts the genetic diver-

sity of offspring. But it's also important to get the chromosomes in position for meiosis.

Meyer and colleagues revealed this January that a protein that's part of the all-important dosage compensation complex has another job—helping govern the number of times crossing over happens. According to Meyer, this link is "completely unexpected" and suggests that crossing over and dosage compensation in worms use a similar molecular mechanism to make big changes to the chromosomes.

As they've investigated the details of dosage compensation, Lee, Meyer, and other researchers have wandered into strange territory. They've come across molecular battles, take-charge RNA molecules, and furtive liaisons between chromosomes. And that's just the beginning. Plenty of unknowns remain. Mammal cells, for example, count their X chromosomes and randomly pick one for inactivation. Nobody knows how they manage either task. Whatever the answers turn out to be, Lee and Meyer say they're expecting more surprises. ■

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before making a broader point. "It's worthwhile to bear in mind," he says, "that we would not have iPS cells except for the ability to study embryonic stem cells. The same people who are now crowing that we don't need embryonic stem cell research tend to forget that we would never have gotten to this point without it."

Advances are coming so quickly that it's difficult to get top scientists to speculate about where the field will be a year from now. Orkin expects that Hochedlinger's

work comparing both types of cells will raise a "cautionary note" for researchers. And Orkin hopes his research will provide the tools needed to create iPS cells that more closely mimic ES cell lines.

Daley is making more iPS cells, creating lines of cells with various blood diseases. In the near term, he hopes that, by transferring diseases from patients into Petri dishes, he'll be able to learn more about disease progression and possibly identify therapies, as he can conduct experiments in cell cultures that he wouldn't do with patients. Looking further

ahead, he remains committed to the possibility of doing for people what he's already done for mice.

"We think that these disease-specific lines will ... help lay the foundation for using genetically repaired cells to replace disease tissues," he says.

Of course, before he can do that, scientists will have to learn to reprogram cells without using viral vectors, a challenge that everyone seems to be pursuing but that no one wants to discuss in any detail. Daley will only say, "That's the hottest area of research in the lab right now." ■



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