Gene by gene, scientists dig for the triggers

January 2 2009, By Mark Johnson

James Thomson knew that to send a cell back to its past was no trivial matter. Like generations of biologists, the University of Wisconsin-Madison stem cell pioneer had been taught that development was a one-way street; it began with an embryo and finished with all the mature cells that make up the body. Yet in the summer of 2007, Thomson and scientists around the globe were racing to do what once had been thought impossible: to reverse the natural process and return old cells to their embryonic origin. They sought the healing potential of embryonic stem cells - immortal in a lab dish, able to become any cell in the body - but without the controversial destruction of human embryos.

An entire field, regenerative medicine, had come of age based on the promise that stem cells might someday offer a basic tool to understand and repair damaged organs and tissue. If scientists could obtain them by reprogramming a patient's own cells, they would bypass two major obstacles: the ethical debate over the use of embryos and the risk that one body will reject transplanted cells from another.

Millions suffering from Alzheimer's, Parkinson's and numerous diseases that have stymied modern medicine would find a new source of hope residing in their own bodies - in something as ordinary as a skin cell.

It was this powerful idea that drove Japanese surgeon-turned-scientist Shinya Yamanaka. Working in secrecy at his lab in Kyoto, Yamanaka and a colleague had discovered a new way to return the adult cells of a mouse to an embryonic state. His announcement in 2006 stunned the field, and he was working furiously to repeat the feat with human cells.

Dozens of scientists were.

The president of the International Society for Stem Cell Research was trying to reprogram human cells in his lab at Children's Hospital Boston. A few miles away, a professor at the Massachusetts Institute of Technology had taken up the challenge. So had two of his former students, one at Harvard University, the other at the University of California, Los Angeles. So had scientists in China, Britain, Germany, Spain and Singapore.

Yamanaka, then 44, knew many of his competitors; some had told him that they were in the hunt. But there was one team he had not taken into account.

In Madison, where he had become the first person to isolate and grow human embryonic stem cells, Thomson, 48, now was attempting to shatter the new biological barrier. He and a postdoctoral student, Junying Yu, had launched their effort in 2003, keeping the project so quiet that other stem cell researchers on campus were unaware of it.

Now, four years into his quest, Thomson knew he was not alone. Sometimes, he thought of Yamanaka. He worried that his rival would announce the discovery before him.

The scientists struggled with the need to be first, and also the need to be sure. Lab lights burned well past midnight.

Some had gone a year without vacation. Then a second year. A third.

They knew a discovery of this magnitude would launch the career of a young biologist, or validate years of toil by a senior researcher.

"Make no mistake, we all knew that was the Holy Grail," said

Massachusetts stem cell researcher Robert Lanza. "We didn't know if it would be found in our lifetime."

The story of how researchers sought to reprogram cells spans more than 25 years and illustrates the conflicting forces at play in science: the incremental steps and the leaps of imagination; the fierce competition and the demand for rigor.

Ultimately, the discoveries - from the isolation of embryonic stem cells to the cloning of Dolly the sheep and cell reprogramming - have been leading toward an extraordinary power.

"I think we have been in a race for decades around the following issue - and I think this is very important: learning how to instruct our own cells to get them to do what we want them to," said John D. Gearhart, director of the University of Pennsylvania's Institute for Regenerative Medicine.

At Children's Hospital Boston, researcher George Daley envisioned a new era.

"We've spent the last hundred years turning chemicals into drugs," he said. "Now we need to be able to turn cells into medicine."

The race began with a discovery: the extraordinary cell that scientists would try to re-create.

In the early 1970s, when American researcher Gail Martin went to work with Martin Evans in London, no one spoke of "embryonic stem cells." Scientists had yet to isolate, let alone name them.

Instead, Evans and others were studying strange tumors called teratocarcinomas, from the Greek word teratos: monster. They contained a variety of tissue - even teeth and bits of bone and hair. Most intriguing

was what lay inside the tumors: primitive stem cells that could develop into other cell types.

Evans, a biochemist at University College London, wanted to grow tumor stem cells, and Martin spent her postdoctoral years figuring out how. Both realized there had to be similar cells in the embryo that could make all the different body parts.

By 1980, Martin was searching mouse embryos for these cells in her lab at University of California, San Francisco. Unfortunately, they were hard to isolate in some strains of mice, and she had not been fortunate in her choice.

To help the cells, she tried placing them in a nutrient material in which tumor stem cells had been growing. They thrived and developed into different cell types.

But there was one last experiment Martin considered vital.

Each embryo cell might have a limited potential. One might form only skin; another, only blood. Martin began growing a single cell to see if it would produce all the different kinds.

She did not realize another scientist was chasing the same cells: Evans, now at the University of Cambridge.

In the summer of 1981, Martin opened the journal Nature to find a new paper by Evans and his colleague M.H. Kaufman. They had cultured the cells from mouse embryos. They called them EK (Evans-Kaufman) cells.

Martin had been working six, sometimes seven days a week, 18 hours a day. She had grown the same cells.

Only to be scooped.

Nonetheless, she finished her final experiment and showed that a single cell from the embryo could give rise to a great variety. A few months later, her paper appeared in the Proceedings of the National Academy of Sciences.

Although Evans and Kaufman had published first, their name for the new cells never caught on.

Martin coined the term that stuck: embryonic stem cells.

James Thomson's goal was clear. In the course of a few days at the Roche Institute, he would follow the lead of Martin Evans and Gail Martin and learn to derive mouse embryonic stem cells.

But the 29-year-old scientist already had set his sights on something more ambitious.

Over a lunchtime beer with his instructor, British developmental biologist Colin Stewart, Thomson revealed that someday he hoped to get these cells from monkeys. Embryonic stem cells from monkeys would offer a much better model of human development than those from mice.

Thomson, who discovered science in the boyhood pursuit of frogs and bugs, had just earned his doctorate from the University of Pennsylvania. He had studied with Davor Solter, a Croatian-born biologist known for his skill with cells and his preference for graduate students who required no hand-holding.

In the lab, Thomson kept to himself, neither collaborating with others nor asking their help, Solter said. The professor saw in his student that scientific temperament. Thomson followed thoughts to their logical

conclusions. He looked for ways to prove or disprove his own ideas. For fun, he played with boomerangs.

In molecular biology, most doctoral students handed in a dissertation of 150 to 200 pages. Not Thomson.

"His thesis was one of the best I've seen, and I've been here 30 years now. It was 59 pages long, as I recall," said Richard Schultz, who led the committee evaluating Thomson's thesis. "He set up a wonderfully clever set of experiments where he could map the fates of these cells, and the results were just crystal clear. Hence a very short thesis."

Although Thomson faced his committee ready for battle, the usual rigorous questioning never took place. There were no questions at all.

At Roche, Stewart had little difficulty teaching Thomson to isolate and grow mouse embryonic stem cells. The following year, Thomson planned to start postdoctoral studies at the Oregon National Primate Research Center, where he could extend his stem cell work from mice to monkeys.

But as they talked over lunch that day, Stewart told Thomson that scientists in Britain were trying to obtain human embryonic stem cells - so far without success.

Thomson had given no thought to working with human embryos, but the goal had a compelling logic. Monkey cells would make a better model of human development than those from mice.

Better still would be the human cells.

Shinya Yamanaka was not on the fast track in his native Japan.

The postdoctoral student from Osaka City University arrived in San Francisco, a bright, mild-mannered man with a gap on his resume. He was not coming from one of his country's top schools or largest labs.

"A promising guy, but he didn't have the gold-plated pedigree that is so important in Japan," recalled Bruce Conklin, an investigator at the Gladstone Institute.

Nor did Yamanaka have a lengthy background in science. His father owned a small factory that made sewing-machine parts. The son had discovered judo and rugby, which set him on a different career path. After suffering broken bones at least 10 times, he decided to become an orthopedic surgeon.

But in residency at a hospital in Osaka, Yamanaka saw patients with severe spinal cord injuries, people no doctor could help. He grew frustrated with the limits of modern medicine and decided to return to a university.

He planned to study science for four years, search for new tools to help patients, then return to the hospital. But he found more freedom in science than in surgery and never went back.

Yamanaka grew interested in techniques that allowed scientists to knock out a harmful gene or insert a beneficial one. Because the technology was not popular in Japan, he applied to dozens of labs abroad and received a fellowship at the Gladstone Institute.

"The boss was very brave to hire me," he said, "because I didn't have a lot of experience in molecular biology."

Yamanaka wasted little time immersing himself in stem cells and other advances in the field. His research focused on one particular gene. By

boosting this gene, he hoped to lower cholesterol in mice.

Colleagues noticed that the young scientist - married with two small daughters - worked late into the night. He spent weekends in the lab. But another quality truly separated Yamanaka from others.

"He was like a heat-seeking missile that can redirect toward something promising," Conklin recalled. "When other scientists would give up, Shinya found opportunity."

His first experiment flopped. The gene that was supposed to lower cholesterol gave the mice tumors.

The other scientists looked at this young man who worked so hard and seemed so bright and thought what a shame that he would return to Japan with little to show.

Although discouraged, Yamanaka was curious. Why had a single gene caused so much damage? He began examining what makes tumors form and what suppresses them.

He wondered if maybe the cholesterol therapy had interfered with a different gene that might prevent tumors.

In late 1996, his time at Gladstone at an end, Yamanaka flew back to Japan. Mice from the lab soon followed on another flight. Busy and with no assistant, Yamanaka had his wife collect the mice at the airport and take them home on the bus.

The scientist tested his hunch by breeding mice and removing the second gene, the one he believed stopped tumors. The embryos died early in the uterus. Without that gene, the mouse's embryonic stem cells had lost their ability to make the other body parts.

He turned to embryonic stem cells.

The very idea of reprogramming cells would have seemed like science fiction if not for a conversation in a Dublin, Ireland, bar.

Ian Wilmut, a young researcher who worked with livestock, was visiting Ireland for a conference in 1986. In the bar, he learned that a Danish scientist had produced live calves using a method in which he removed the nucleus from an unfertilized egg and inserted in its place the nucleus from an early embryo.

If the unpublished Danish work was correct, Wilmut realized he could create calves and use the process to introduce disease-resistant genes. Scientific dogma could be overthrown.

"It had always been said that cloning was impossible to do in mammals, but I didn't believe it," said Keith Campbell, who worked with Wilmut at the Roslin Institute in Edinburgh.

More than a half-dozen Roslin staff members took part in the four-year mission to clone a mammal. The sheep egg was small, the process inefficient. Researchers removed genetic material from the egg and inserted the nucleus of a more mature cell. They cloned more than 200 embryos, but only 29 developed sufficiently to be transferred to ewes.

It took 40 days to confirm a pregnancy and another 100 to learn whether the pregnancy would yield a live birth.

For weeks, researchers slept near the pregnant sheep, ready to call a veterinarian if a problem arose.

In July 1996, while vacationing, Campbell called the lab for a progress report and heard excitement on the other end. The first cloned mammal

had been born, Lamb Number 6LL3. Cloned from a cell in the mammary gland, the lamb was dubbed Dolly, after Dolly Parton.

Wilmut and Campbell made sure the animal was healthy, then in February 1997, they announced the historic birth to the world.

The cloning of Dolly left a lasting impression on many scientists, including Shinya Yamanaka in Japan and James Thomson, who by then had joined UW. The principal lesson they took from the experiment was not about making exact copies of living creatures, the vaguely creepy notion lodged in the public imagination. For biologists, Dolly offered an alternative to the one-way street from embryo to maturity.

"That showed the arrow of time is not irreversible in development," Thomson said.

At a slide presentation in Washington that June, Wilmut predicted where his discovery would lead. Scientists, he said, will find a way to put key factors into cells and reprogram them.

"I've still got the slide," he would say a decade later.

James Thomson had the ideal job for a media-shy scientist.

As chief pathologist at the Wisconsin National Primate Research Center at UW, he performed necropsies on monkeys, work unlikely to attract TV cameras. In his spare time, he enjoyed hang-gliding with its "man-against-nature" thrill.

But Thomson's life outside the spotlight was coming to an end, and he knew it.

For more than a year, he had been starting work early, at 5 a.m. Instead

of reporting to the primate center, he arrived at a lab next to the university's in vitro fertilization clinic. For several hours, he would work with human cells, before rushing off to his day job.

Between 1992 and 1995, Thomson had achieved his goal of obtaining embryonic stem cells from monkeys - marmosets and rhesus monkeys. The work marked a significant advance toward the next step: human embryonic stem cells.

It was not a step he took lightly.

"I wanted to first decide that I could do this in my own mind," he said, "but also whether it was worth all of the grief, all of the publicity it was going to generate."

He thought about the knowledge that could come from a human embryo. And he thought hard about using that embryo "as a utilitarian object."

"If you don't find it at least a little bit creepy," he said, "you haven't thought about it enough."

For Thomson, the deciding factor was this: The embryos he planned to use would never become babies. They had been made by couples who had opted not to use them.

In vitro fertilization, which had been in its infancy in 1981, now was in common use by infertile couples. Typically, couples produced more embryos than they needed and chose the best; the rest were frozen.

Although Thomson now had decided he could work with human embryos, he did not proceed immediately. Instead, he consulted two UW ethicists: Norman Fost and Alta Charo.

In 1994, in the midst of his monkey research, Thomson met Fost in a campus library and explained the project he was planning.

"It wasn't that he wanted to know how to spin it or how to get through whatever regulatory hurdles there might be," Fost said. "He said over and over again: 'I want to get it right. I want to know what the right thing to do is.' "

Informal meetings with Fost and Charo were followed by a formal application to the UW board that evaluates all human-subject research. Quietly, the board began examining the work in extraordinary detail. Eventually, a bioethics advisory committee was appointed solely to prepare for the issues raised by human embryonic stem cells, including the sensitive matter of destroying embryos.

In 1996, Thomson and his colleagues took their first steps, identifying human embryos of the right quality and developing techniques to isolate the stem cells within. The embryos were tiny - the size of the diameter of a human hair. The human cells were harder to grow than those in mice and harder to keep from developing.

But early in 1998, Thomson's team achieved a breakthrough. It began when scientists in Australia, seeking to improve in vitro fertilization, arrived at a crucial insight. Conditions in the embryo's first three days were vastly different from those in the next few. Embryonic tissue became more active, a shift supported by a change in nutrients.

The Australian team tried moving embryos after a few days to a different nutrient material in order to mimic the changing environment in a woman's body. The proportion of embryos surviving to the 5-day-old stage more than doubled.

In Madison, Jeff Jones, one of the scientists working with Thomson, also

directed UW's in vitro fertilization clinic and realized the two-stage system might prove critical to their project.

"The first time we put the embryos in this system, what they looked like, it was just night and day," Jones said.

Poor-quality embryos with few cells were replaced by healthy embryos with many. Now the scientists had to prove these were embryonic stem cells. The cells would have to survive for long periods and develop into many different types.

After a few months, Thomson peered into the microscope and saw tiny cells that did not lie still. They pulsed. They had become early beating heart cells.

"That's when we knew we had the right thing," he said.

At Johns Hopkins University School of Medicine, scientist John Gearhart had been searching human fetal tissue for something similar. The primordial germ cells he obtained normally became sperm or eggs, but in a culture dish, some changed and gained the power to become any cell in the body.

By fall, Gearhart had submitted a paper on the special cells to Proceedings of the National Academy of Sciences. Then, the journal Science contacted him to ask if he might review a forthcoming paper.

The first author: James A. Thomson. The Madison group had isolated human embryonic stem cells.

Gearhart told the Science editors that he had submitted a paper to another publication announcing a similar discovery. Later, he would come to believe this disclosure roused the editors at Science.

Thomson's paper appeared on Nov. 6, 1998; Gearhart's came four days later.

"Look, I'm not complaining in any way," Gearhart said this spring. "It was just one of these interesting events that happened along the way."

As James Thomson had predicted, the discovery raised new scientific possibilities, but came at the expense of his privacy. The quiet researcher, a man who did not own a television, would be interviewed by reporters, confronted by protesters and photographed for the cover of Time.

And he would realize very early that there might be a way to pull together the decade's two biological breakthroughs: embryonic stem cells and cloning. Cloning had shown that development could be reversed, so it ought to be possible to make mature cells become embryonic.

In 1999, a post-doctoral student in Thomson's lab began work on cell reprogramming. She made little progress and eventually left the lab. Reprogramming was a monumental task, and Thomson lacked the right researcher for it.

By the time he found one, Shinya Yamanaka would be two years into his own quest to send a cell back to its past.

Back in Japan after his fellowship, Shinya Yamanaka struggled. The 36-year-old scientist found the facilities at Osaka City University so much worse than those he'd enjoyed at the Gladstone Institute of Cardiovascular Disease in San Francisco. He wondered if he ought to return to his old job as a doctor. At least then he had known he was helping someone.

In San Francisco, Yamanaka had risen for work every morning at 5 a.m. He was in the lab by 7. But in Osaka, he slept until 9 a.m., sometimes 10. His wife began to worry.

"I almost gave up being a scientist," Yamanaka recalled. "I couldn't concentrate on experiments, and I wasn't sure there was any meaning continuing such research in a poorly funded old building."

Then, at the end of 1999, he moved to a new job at the Nara Institute of Science and Technology. He found himself working in better buildings, surrounded by good scientists and promising students. His ambition returned.

He remembered that the previous year, he'd read James Thomson's paper describing human embryonic stem cells. The discovery appealed to the doctor in him.

"It may rescue many, many patients," Yamanaka said.

When he learned that Japanese scientists were having trouble obtaining human embryos, he even considered suggesting to his wife that they donate their own. But he never did have that conversation with her.

A short time later, Yamanaka went to see a friend who worked at a fertility clinic. The friend let him view embryos under the microscope.

"The difference between my daughter and these tiny cells looked very small to me," Yamanaka said. "I can imagine if we transplant this embryo, it may become just like my daughter, or I could destroy this and try to make human embryonic stem cells. So the decision is very tough."

He could not stare through the microscope purely as a scientist or purely as a father. When he looked at the cells, he was both.

Yamanaka concluded that if there were no other way to cure a patient, then the cells from the embryo should be used. Still, he thought it would be better if science could harness the same healing potential without the loss of an embryo.

What if there were a way to turn a mature cell into an embryonic stem cell? Yamanaka understood the difficulty of what he was suggesting. Dozens of genes might have to be inserted into a cell in precise amounts and in a specific sequence.

But in 2000, when he set out to try, the struggles in Osaka were still fresh in his mind. He remembered the meaning draining away from his work.

"So as a scientist, I died once already," he said. "I thought, 'I can always die as a scientist. So let's do something important and very risky.' "

On her way back from a formal interview at the University of Minnesota, scientist Junying Yu decided to stop in Madison, Wis. The reason was James Thomson. She hoped an interview with the stem cell pioneer might lead to a job offer.

Because he was on vacation, she spent an extra night in Madison.

Yu was the daughter of poor, illiterate rice farmers from eastern China. Her parents had little knowledge of science and no interest in her work. But in school, she always had managed to find teachers who encouraged her.

Like Thomson, Yu was quiet and methodical.

The similarity struck her adviser at the University of Pennsylvania, Richard Schultz, who had led the committee evaluating Thomson's

doctoral thesis. In Schultz's lab, colleagues had given Yu the nickname "La Machine." She arrived around 8 or 9 each morning and departed at 7 in the evening, leaving a spotless lab station.

"You would look at her bench and you would think, 'Does anyone work here?' " Schultz said.

Her studies left no doubt: complex sets of experiments performed not just once, but three times. There were six published papers, too, three with a short list of authors: Yu, Schultz and another professor.

"That meant basically she did all of the work," Schultz said. "You very seldom see this, a paper with so few authors on it."

After staying overnight in Madison, Yu had her interview with Thomson, now an assistant professor. She talked about her fascination with reprogramming, an interest he shared.

The hiring decision was not difficult. Yu arrived in Madison the following February and began work on cell reprogramming, the project that had been suspended in 1999.

"The feeling was that this was a 20-year project," Thomson recalled. "She'd get a nice start on it and find some interesting things, but wouldn't actually accomplish it."

Shinya Yamanaka wondered: What keeps a cell in the embryonic state? And what releases it on the path toward becoming a bit of bone or brain?

To send a cell back to its primitive origin, he would have to understand what was different about an embryonic stem cell, what gave it the potential to contribute to any part of the body. Scientists call this potential pluripotence.

They knew some genes that played an important role, including two called Oct4 and Sox2. And in late 2002, Yamanaka and his students were closing in on another.

His team compared the genes that are switched on in mouse embryonic stem cells with those in more mature cells. Yamanaka wanted to know which genes were turned way up in the beginning, then shut down as the cell developed. Eventually, the scientists focused on eight.

In order to learn which of the eight genes was most important, they made different groups of cells, then removed a critical factor from all of them. Without this factor, cells with seven of the genes left the embryonic state. One gene, however, kept cells young. Yamanaka called it Ecat4.

A team at the University of Edinburgh in Scotland discovered the same gene at roughly the same time. The Scottish scientists, led by Austin Smith, inserted the new gene into mouse embryonic stem cells. They discovered that when they tried to force cells to mature and leave the embryonic state, those with the special gene stayed.

The Scottish team named it Nanog, from Tir nan Og in Celtic mythology. The land of the ever young. Nanog was no ordinary gene; Smith compared its role in an embryonic stem cell to that of a conductor in an orchestra.

Yamanaka and the Scottish researchers shared the discovery of Nanog, publishing separate papers in May 2003 in the journal Cell. In his paper, Yamanaka made it clear where the new gene was leading him. Embryonic stem cells had inspired hope as a possible treatment for diseases, but their use had raised ethical issues, he and his colleagues wrote. "One solution to avoid such ethical issues is to generate pluripotent cells" from mature cells. In other words, Nanog might help researchers create an embryonic stem cell free from ethical objections.

A good cell.

While scientists praised the discovery of Nanog, few made the leap to reprogramming.

"No. I remember reading that and thinking, 'This guy really is crazy,' " said Smith, who had befriended Yamanaka. "It's not the way people were thinking about it at all."

With a notable exception.

"As we know more and more about pluripotency," James Thomson told The Washington Post, "it probably will be possible to reprogram cells to make stem cells out of any cell in the body."

Before almost anyone else, Thomson and Yamanaka had envisioned where their field was headed.

Yamanaka's paper listed eight co-authors, including one of his first students at the Nara Institute, Kazutoshi Takahashi. The son of a businessman and a pharmacist, Takahashi admired his teacher's earnest manner and adventurous spirit. The student's loyalty was absolute.

"The most important thing for me is to work with Dr. Yamanaka," he said in an e-mail. "If Dr. Yamanaka will go to another scientific field, I will follow him."

In 2003, Yamanaka wrote a risky grant proposal. A long shot. He wanted to reprogram mouse cells.

Most grant reviewers never would have funded the experiment for a simple reason, Smith said. "It shouldn't work."

Nonetheless, Yamanaka brought the proposal to Tadamitsu Kishimoto, the former president of Osaka University and one of Japan's most famous scientists.

"So, this is a kind of dream. This was his idea," Kishimoto recalled. "But nobody believed it. I did not believe it."

Still, if the experiment failed, Kishimoto thought the young scientist might discover something interesting. He gave Yamanaka a grant of $600,000 a year for five years.

Yamanaka decided to tackle reprogramming with one other scientist in the lab, someone who shared his fondness for risk: Takahashi.

They kept the project secret - even from fellow lab members.

Junying Yu was under no illusions.

Her reprogramming project likely would fail. Still, Yu believed she might gain valuable insight into the different genes and their roles.

Although she would be supervised by one of the field's luminaries, James Thomson, she would carry out most of the hands-on lab work herself. Many of the key day-to-day decisions would be hers.

Years ago, Thompson explained: "I derived human embryonic stem cells with my own hands. I did it myself. That's not true in my lab anymore."

He had been promoted to full professor after just three years, bypassing the traditional rank of associate entirely. Like other established scientists, he now directed a lab that included more than a dozen graduate and postdoctoral researchers. His job was to come up with ideas, secure grant money and see that research turned into publications.

Years ago, his own adviser Davor Solter had given him the freedom to take the lead on projects and grow as a scientist. Now Thomson played a similar role with Yu, meeting every couple of weeks to discuss direction and resolve problems.

She began with an experiment to demonstrate that reprogramming was possible. She fused embryonic stem cells with primitive blood cells. The blood cells traveled back to an embryonic state. Although the procedure would raise familiar ethical issues about the use of embryos, it was revealing.

The power to reprogram lay inside an embryonic stem cell. What did it have that a blood cell did not?

Yu compared the genes switched on in blood cells with those turned on in embryonic stem cells. She found 250 genes that were activated in embryonic stem cells, but not in blood cells.

Somewhere among all the potential groupings of the 250 genes - the number of possibilities is 76 digits long - there had to be one that turned back the developmental clock.

In October 2005, she published the cell fusion results online in the journal Stem Cells. A Harvard University group had done similar work and submitted its paper eight days earlier. The Harvard paper was published in Science. It was, in Thomson's view, a lesson in the importance of being first.

"You come in second, and you get a little paper in a not-terribly-high-impact journal," he said. "And when you're trying to get a job, it matters. It's unfortunate, but that's the way it is."

Yu, now an assistant scientist, focused on her goal. She dropped the list

to 150 genes by cutting those less promising. Then, by reading the literature and noting all that had been learned about human embryonic stem cells, she prioritized her list of genes. She focused on those that were turned way up or demonstrated an important role in an embryonic stem cell.

In January 2006, the field of 150 became a much smaller group.

Just 14.

Junying Yu did not know she was in a race.

But months before she arrived at her 14 genes, Shinya Yamanaka had reached his own number. Now working at Kyoto University, he and Kazutoshi Takahashi had settled on 24 genes that kept embryonic stem cells from losing their potential. Their list and Yu's only partially overlapped.

Unlike Yu, the Japanese scientists were not working with human cells. They inserted their candidate genes one by one into mature mouse cells. They found that no single gene worked.

Then, in 2005, they infected the mouse cells with viruses carrying all 24 genes. Because of their ability to get into cells, viruses made a good vehicle for delivering the genes.

The process did not work on the vast majority of cells, but when the scientists examined the culture dishes closely, they discovered a scattering of tiny cell colonies. They resembled embryonic stem cells.

Yamanaka allowed himself to think for a moment: Wow, it may have worked.

"But at the same time, I thought this must be some kind of mistake," he said, "because that happened many, many times in my life as a scientist: We got excited, and we found out it was some kind of a mistake."

Takahashi repeated the experiment the next day and many more times in the weeks that followed.

Yamanaka worried. What if the cells had been contaminated? It would take only a few embryonic stem cells mixed in with the others to explain the colonies they were seeing.

Yamanaka had another researcher repeat the experiment. Again, it worked.

A critical task remained before the results could be published. Yamanaka and Takahashi would have to document which of the 24 genes were truly necessary to reprogram a cell.

Takahashi devised a methodical approach. They would subtract one gene from the 24. If reprogramming still worked well, the subtracted gene was unnecessary. If it failed, the gene was important. They whittled their candidates down to 10, then went through a second round of elimination.

The conventional wisdom was that reprogramming a cell would require dozens of genes.

In the end, it took just four.

They would become known as the "Yamanaka factors": Oct4, Sox2, Klf4 and c-Myc. Two of them, Oct4 and Sox2, were obvious choices; the omission of one, Nanog, was surprising.

Yamanaka and Takahashi found the simplicity shocking. They repeated

the experiment 10 to 20 times with the four genes until they were certain.

In March 2006, at the Keystone Symposia in Whistler, British Columbia, Yamanaka dropped a bombshell.

He told the audience, which included James Thomson, that his team had reversed development. They had done it with four genes. And they were attempting the same feat with human cells.

But Yamanaka left researchers in suspense. He would not name the genes.

"The method was too simple," he explained later. "Everybody can do it within a week. ... We knew that it was a very important discovery, but at the same time, because of the simplicity of the method and because of the significance of the result, I thought many people would not believe the result."

Scientists around the globe did find it hard to believe. How could something they assumed to be so difficult turn out to be so simple? Torn between euphoria and doubt, they waited for Yamanaka's paper.

"The network of scientists was such that we knew a couple of the genes," said John Gearhart, the John Hopkins University scientist who had competed with Thomson in 1998.

"But we were wondering, 'What's the third gene? What's the fourth gene?' It was a drama that went on for days."

Junying Yu now assumed she had no chance. Her scientific rival in

Japan, Shinya Yamanaka, had sent mature mouse cells back to their embryonic origin. All that remained was for the work to be published. Soon he would do the same with human cells.

In 2003, Yu and her supervisor, University of Wisconsin stem cell pioneer James Thomson, had set out to reprogram human cells, unaware that Yamanaka was chasing the same improbable goal. If they succeeded, the scientists would capture the power of human embryonic stem cells without the ethically contentious destruction of embryos.

But Yu's project had been slowed by problems with the culture medium, the nutrient material the Thomson lab was using for cells.

"It basically wiped out everybody's experiments," she said.

Despite her fear that Yamanaka was now far ahead, she pressed on.

"Everything else in my life," Yu said, "simply appeared insignificant."

Her parents, poor rice farmers in China, had never shown an interest in science. Over the years, she had learned to rely on a strong inner navigation.

It was time to test the 14 genes she had selected as the best candidates to reprogram a cell.

Using viruses to deliver the genes, she inserted all 14 at once into human cells. On the morning of July 1, 2006, Yu arrived at the lab and examined the culture dishes. Her eyes focused on a few colonies, each resembling a crowded city viewed from space. They looked like embryonic stem cells.

The excitement left her so distracted that later she locked herself out of

her apartment.

When Thomson returned after the long weekend, he inspected the lab dishes. Years of working with embryonic stem cells had made a skeptic of him. Just because they looked like embryonic stem cells did not mean they were.

Cells must pass certain tests. They must multiply for weeks while remaining in their delicate, primitive state. When they are allowed to develop, they must turn into all the other cell types.

Bad things happen. Cells develop too soon. Cells die. There is no "aha!" moment, Thomson has said, only stress.

He looked at the colonies and suppressed any excitement. He told Yu, essentially: OK, well, get back to me in a couple of weeks.

One month later, Yamanaka's mouse paper appeared online in the journal Cell.

"It was clearly extraordinarily important work from the day it was published," Thomson said.

"We realized that he beat us. She had those 14 factors working prior to that publication, so we kind of had to scrape ourselves off the floor and say, 'OK, at least it's not the human cells.' "

Science has not had an easy time translating breakthroughs achieved in mice to humans. After the isolation of mouse embryonic stem cells, it was 17 years before Thomson obtained the human equivalent.

The physiologies of mice and humans differ significantly. Mouse cells divide once every eight hours; human cells take much longer, one to two

days. An experiment may require two weeks with the cells of a mouse, but two to three months with those of a human.

Yu and Thomson were behind, but they had one advantage: They had the human cells.

Shinya Yamanaka had been right to worry that his reprogramming results would not be believed.

Skepticism persisted, even after his paper was published. Although the reprogrammed cells appeared similar to embryonic stem cells, Yamanaka had noted that one experiment failed.

To demonstrate that a cell can make not just many but all the different varieties, scientists insert it into a mouse embryo to see whether it will contribute to an entire baby mouse. These mice, called chimeras, are one of the key tests to determine an embryonic stem cell. Yamanaka and fellow researcher Kazutoshi Takahashi were unable to make a chimera with the reprogrammed cells.

Some voiced doubts, but not British scientist Austin Smith.

"Yamanaka's a friend of mine, so we'd discussed these results, and it was absolutely clear what he'd done and that he hadn't gotten it all the way, but that he would," Smith said.

Yamanaka now wanted to be the first to reprogram human cells. He also wanted to answer the skeptics and achieve complete reprogramming with mouse cells. In both tasks, he faced competition.

While scientists prefer to pursue their own experiments rather than confirm work by others, Yamanaka's discovery was different.

"It was such a fundamental finding that proving or disproving it was motivation enough and reason enough to do it," said Konrad Hochedlinger, then at the Whitehead Institute for Biomedical Research in Cambridge, Mass. "With all of these follow-up ideas and projects in mind, we felt we just had to reproduce it."

In February 2007, Yamanaka submitted a new paper to the journal Nature. Using a slightly different technique, he generated cells that passed the chimera test. A few weeks later, a group that included Hochedlinger and Rudolf Jaenisch at the Whitehead Institute submitted a similar paper to Nature. Then the journal Cell Stem Cell received a third related paper by a group including Kathrin Plath of University of California, Los Angeles.

All three studies proving that reprogramming worked were published June 6, 2007.

"They were all done a little bit differently but basically the same conclusion," Jaenisch said. "Nobody could doubt anymore."

George Daley did not want to get drawn into a race.

Daley, the president of the International Society for Stem Cell Research and a scientist at Children's Hospital Boston, had approached reprogramming from his own direction. His lab was attempting to add genes to germline stem cells, which make sperm. They hoped to convert these into embryonic stem cells.

When Shinya Yamanaka's 2006 paper was published, the Children's Hospital team tried using his reprogramming technique on the germline stem cells. It didn't work. That left Daley with the question he knew the other labs would be racing to answer.

"So I reluctantly allowed my postdoctoral student to ask whether you could do these experiments in human cells," he said, referring to Yamanaka's mouse experiments.

After trying without success to reprogram adult cells, the lab switched gears and in the spring of 2007 applied the technique to much younger cells. Once they got the method working, they went back to adult cells.

Using the same genes as Yamanaka, the Children's Hospital researchers reprogrammed a variety of human cells, including some from a skin biopsy. Now they faced the hard work of proving their results.

"I was very insistent that we do a couple of experiments to really nail down the biology," Daley said.

The experiments gave them confidence, but at a price: several extra months in the midst of a fierce race.

Although Daley's group had reprogrammed human cells, it wasn't long before others in the area had, too. Within a few miles of Daley's lab, two other scientists had been attacking the problem.

Rudolf Jaenisch, who had spent years studying the technique used to clone Dolly the sheep, had entered the race later than Yamanaka and James Thomson. But his team at the Whitehead Institute progressed rapidly. By September, he had the human cells.

His former student Konrad Hochedlinger, now at Harvard University, had run into problems with a bacterial infection that tainted his cell cultures. Yet by September, he, too, had succeeded.

To reach the scientific Holy Grail, they would have to prove, then publish, their results.

The Boston groups were not the only ones the scientists in Madison, Wis., and Kyoto had to worry about.

A team led by Kathrin Plath and William Lowry was reprogramming human cells at UCLA. Plath, a former student of Rudolf Jaenisch's, now was an assistant professor with a small lab of her own.

Day after day, she and postdoctoral student Rupa Sridharan toiled in the lab until 2 or 3 in the morning.

"We knew it was a race and all hands on board," Sridharan said.

By July, Plath said, they had the human cells.

But were they equivalent to embryonic stem cells? For ethical reasons, scientists could not test human cells by making chimeras. Instead, they inserted the cells into special mice to see if they would generate teratomas, a type of tumor that has cells from the three germ layers that make all human organs - endoderm, mesoderm and ectoderm. A cell that can trigger such a tumor presumably can give rise to all the cells in the body.

Plath waited for the mice to develop tumors.

Junying Yu and James Thomson had reprogrammed human cells in 2006 - long before the teams in Boston and Los Angeles. But before they could announce the achievement, they needed to establish which of the 14 genes they'd used were essential. And they needed to show that the new cells acted like human embryonic stem cells.

In the fall of 2006, Yu was preparing to whittle down her list of genes when she fell ill. The pain in her gut was awful. She struggled to eat. Her doctor thought it was stomach flu.

Instead, in late October, Yu's appendix burst. She was laid up for a month. When she returned to the lab, the problem with the culture medium struck again.

Not until January 2007 was she able to begin narrowing the list of genes. She spent several months testing subsets of them, finally arriving at four. Two, Oct4 and Sox2, were "Yamanaka factors," the name given to the genes Shinya Yamanaka had used to reprogram mouse cells. Two, Nanog and Lin28, were not.

Using a virus to deliver the four genes, she reprogrammed a line of fetal cells, then repeated the experiments with more mature cells. Although the process was inefficient, succeeding with only a small fraction of cells, it did work.

The simplicity made her uneasy.

"There weren't any magic factors that people didn't know," she said.

All year, Yu had been keeping a close watch on stem cell papers, looking for clues as to who was in the hunt, who might be close.

In August, Nature Biotechnology published a paper online from Rudolf Jaenisch's lab. Suddenly, Yu grew nervous. Earlier in the year, she had discovered a simpler way to isolate the reprogrammed cells, an important step because less than 1 percent successfully return to the embryonic state. She had not published this work because she thought it gave her an advantage over other groups.

Now Jaenisch's team had accomplished the same thing. Yu and Thomson discussed their project and raised the possibility that another group already might have submitted a similar paper to one of the journals. Thomson was no newcomer to scientific competition, but Yu could tell

he was worried.

They began writing.

The Madison scientists planned to split their four-year project into two papers. They submitted one to Nature, describing the process by which they'd screened their original 250 genes to reach the final four. Yu said Nature accepted the paper for review, a first step that signaled the journal had not received anything similar.

The second paper, which arrived at Science on Oct. 9, described the actual reprogramming of mature cells. It, too, was accepted for review. After all the anxiety, this response from two leading science publications seemed encouraging.

Then, to their surprise, Nature rejected the paper.

The journal would not comment on the decision.

"It's typical that things get rejected on first pass," Thomson said. "But that they made no great attempt to accommodate us was surprising. So we gave it to Science."

Forced to fold both papers into a single submission, the Madison scientists boiled down much of the detail describing how they had arrived at the four genes. Unlike the other groups, which had plugged in the four Yamanaka factors, Yu had conducted her own time-consuming analysis. Thomson thought it a shame that the paper might now leave the impression that they had simply followed Yamanaka's lead.

Still, Science was reviewing their work.

Perhaps they had arrived at the finish line first.

Where was Shinya Yamanaka?

By fall 2007, at least five other teams had reprogrammed human cells, though few knew of the accomplishment. Not a word had been published.

Junying Yu sensed other scientists might be closing in, but it was Yamanaka who worried her.

His team had enjoyed a considerable head start. Even before the mouse paper was published in August 2006, the Japanese researchers had begun working on human cells. It wasn't long before they succeeded, though Yamanaka declined to say exactly when because the question relates to patent applications.

"Soon after we started the human project," he said, "we learned that we can do it."

Like Yu, Yamanaka was looking over his shoulder for signs that another scientist might be ready to publish. His concern peaked in the fall of 2007.

"I heard a rumor that many people were about to submit their papers," he said, "so that's why we also submitted our paper."

A few weeks after James Thomson and Yu had delivered their research to Science, Yamanaka and his team took theirs to Cell.

Yamanaka's collaborator on the project, Kazutoshi Takahashi, now an assistant professor, had gone three years without a vacation. There'd been no vacations for Yu either, not in two years. All the work had come down to a final sprint.

On Nov. 12, Cell accepted the paper from Yamanaka's group.

On Nov. 14, Science accepted the paper by Yu and Thomson.

On Nov. 16, Nature received a third reprogramming report, this one from George Daley's group.

On Nov. 17, before any of the work had been published, international headlines reported a huge scientific convert to the new reprogramming technique. Scottish scientist Ian Wilmut announced that after more than a decade of work on cloning, he would abandon the practice and adopt Yamanaka's method.

Editors at Science and Cell had learned that they were about to report a similar scientific finding by two groups half a world apart.

"Editors learned about the other paper in Cell pretty late in the game," said Ginger Pinholster, a spokeswoman for Science.

By e-mail, Cell Editor Emilie Marcus said the journal put Shinya Yamanaka's paper "through our normal peer-review process and set the publication date independently and without any specific knowledge of the timing of competitive papers at other journals."

In the end, Cell and Science agreed to a joint announcement, crediting both groups with the discovery.

On Nov. 20, James Thomson and Shinya Yamanaka, shy men more at home in the lab than in the media, shared headlines around the world. They had known for about a week that their race would end in a tie. Despite the competitive nature of the field, each acknowledged the important role played by the other.

Yamanaka said that without Thomson's isolation of human embryonic stem cells, scientists would not have known how they were supposed to look and behave, and what culture conditions they required. Thomson credited the Japanese scientist for his breakthrough in reprogramming mouse cells.

"It's kind of good karma," Thomson said of the announcement, "as much as we wanted to beat him."

Groups that had opposed research requiring the destruction of embryos hailed the new technique, which avoided the embryo entirely. At the National Catholic Bioethics Center, Father Tadeusz Pacholczyk said he saw "no fundamental ethical problems."

However, scientists cautioned that many questions remained about reprogramming, and they urged that work on human embryonic stem cells continue. The Vatican restated its emphatic opposition to such research, "not only because it lacks the light of God, but also because it lacks humanity."

There would be no tidy end to the ethical debate.

Like others in the field, George Daley praised the breakthrough as enormously important, though some sense of personal disappointment was inescapable. Scientists in his lab had reprogrammed human cells, too; had written and submitted a paper of their own.

"You know, I have to say that in retrospect, I kind of kick myself because I think we could have hurried out a very quick paper in August or September and been ahead of the other groups," Daley said. "But if you look at our paper (published online Dec. 23 in Nature), we ended up reprogramming a much larger array of cell types. ...

"Science is always a balance of wanting to do things deep and thorough and with great confidence and wanting to be first."

The UCLA team, which had finished a draft of its paper, met on the day of the announcement. The researchers talked about what they might have done to avoid finishing behind the other groups. Still, colleagues told them that they had done something incredible.

Talk turned to: What do we do next? How do we get this published?

"You just have to move on," Rupa Sridharan said.

The UCLA group's work was published in February in the Proceedings of the National Academy of Sciences.

Soon after these first papers, dozens of labs around the world were reprogramming human cells. Science adjusted to a new sense of what might be imagined.

Shinya Yamanaka's lab held no formal celebration. Although the cells were a promising tool for studying diseases and testing drugs, they were unsafe for use in humans. The reprogramming method - viruses carrying genes - could lead to cancer. Also, one of Yamanaka's factors was c-Myc, a cancer-promoting gene.

As the former doctor shuttled between his lab in Japan and a second at the Gladstone Institute of Cardiovascular Disease in San Francisco, he focused his attention on making the cells safe.

"I don't think we are ready to celebrate," he said. "It's still a long way to go."

In less than a year, Yamanaka would find a safer way to reprogram cells

- without using a virus.

For his part, James Thomson stressed the immediate value of the new cells.

A skin biopsy from a patient with Alzheimer's or Parkinson's now offered a harmless way for scientists to study how diseases assault our cells and to build new defenses. Researchers could send the skin samples back to the embryonic state, then grow them into the specific cells damaged by a disease. They could watch these cells deteriorate in a lab dish and test hundreds of drugs to see if any could rescue them.

Still, inserting the powerful new cells into people remained a distant frontier.

A few days before the reprogramming discovery was announced to the world, Thomson and members of his lab gathered to mark the achievement. He purchased a bottle of expensive champagne, Dom Perignon, 1998. The year he isolated human embryonic stem cells.

Afterward, Junying Yu took the empty champagne box to her office and placed it on a shelf.

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