FINANCIAL TIMES & SCIENTIFIC AMERICAN

SPECIAL REPORT

The Future of STEM CELLS

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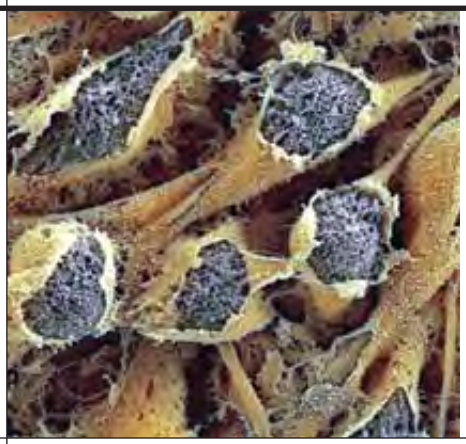
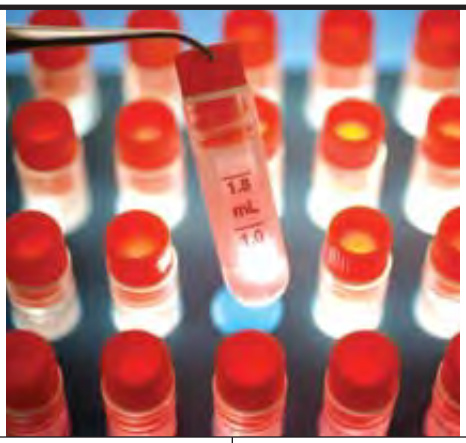
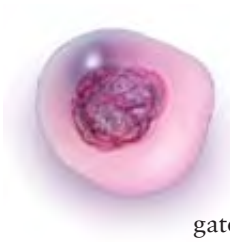
SCIENTIFIC AMERICAN

Contents


Cover montage: Tom Draper Design. Cover images: Corbis (*currency*); Laguna Design/Science Photo Library (*stem cells*); Peter Macdiarmid/Reuters/Corbis (*test tube*); Tom Draper Design (*globe* and *graph*); Q.-L. Ying and A. Smith, Wellcome Photo Library (*neurone network*). Images above: Simon Fraser/Photo Researchers, Inc. (*SEM of stem cells*); Peter Macdiarmid/Reuters/Corbis (*test tubes* and *stem cell bank*)

From the Editors



Stem cells have moved from biological obscurity to the forefront of political and technological debate in the US and around the world. Investigators are confident that someday stem cells will be the foundation for fantastic cures and therapies. Yet critics argue that stem cell research raises ethical questions no less profound than the pursuit of the nuclear bomb more than 60 years ago.

The complexity of the science and the rapid proliferation of business, ethical and political issues pose a challenge for anyone wishing to stay well informed on this vital subject. This is why we believe that stem cells represent an ideal opportunity for an editorial collaboration between the *Financial Times* and *Scientific American*.

This special report draws on the *FT*'s strength in international business and political reporting, which in turn complements *Scientific American*'s long experience in rendering scientific discussions clearly and authoritatively.

It is easy to forget that stem cell research is relatively new. Only in 1998 did scientists first identify and isolate stem cells from human embryos. Today stem cell research has opened a window of opportunity for countries looking to close the customary US lead in biotech. It has reheated discussions of whether and when human rights should inhere in embryos. It has inspired entrepreneurs and spawned new consumer services: prospective parents now routinely receive appeals to freeze the stem cells in their newborns' umbilical cord blood as a hedge against future medical needs.

Such practices have revealed to the public how unsupervised and ethically unguided some practices in fertilisation clinics have been for years. They have provoked a fiscal mutiny of sorts among American states against limitations on federal research funding. They have suggested new forms of fraud: patients in Russia have been victimised by beauty parlours promising that their "stem cell injections" could treat a variety of ills. And, of course, they have raised much technical speculation about the degree of versatility in various types of stem cells and what that may tell us about the latent capabilities of all our tissues.

Virtually no matter touched by stem cells is yet settled. Rather than spelling out final answers, this report should serve as a concise reference on the most important questions to be addressed in the years to come. Both the *Financial Times* and *Scientific American* will continue to provide first-rate coverage of the ongoing evolution of these matters—including, one hopes, the eventual news that stem cells have turned into a stable, reliable source of both practical therapies and financial opportunities.

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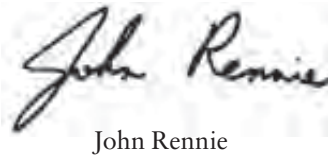

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ANDREW SWIFT

Mother of All Cells

Scientists expect enormous benefits for humankind from the surge of research on embryonic stem cells. But it could take a generation or two before the full impact is felt. Clive Cookson discusses the issues

The late 1990s was the most productive period in the history of biological research. The birth of Dolly, the first cloned mammal, was quickly followed by the first successful derivation of human embryonic stem cells and then, as the new millennium dawned, the completion of the Human Genome Project.

Since then the media have amplified these achievements, with the enthusiastic encouragement of many of the researchers involved, to create intense public excitement about a new era of regenerative medicine. Some people imagine that within a few years it will be possible, through some still obscure combination of stem cells, cloning and genetic engineering, to create new cells and eventually whole organs to replace those that fail through disease, accident or old age.

That promise is counterbalanced by ethical and religious objections to stem cell research—particularly to the idea that embryos could be created especially for research and then destroyed—and fears that therapeutic cloning could open the door to reproductive cloning.

For many people the very phrase "stem cells" sums up all the excitement and fears. But there is widespread ignorance about stem cells and wishful thinking about how quickly their potential will be achieved. This report is intended to shed scientific light on the future of stem cell research—and the associated policy issues that are driving national and state governments to commit billions of dollars of public funds to the field.

First, then, some basic definitions. Stem cells serve as a biological repair system, with the potential to develop into many types of specialised cells in the body. They can theoretically divide without limit to replenish other cells. When a stem cell divides, each daughter can remain a stem cell or adopt a more specialised role such as a muscle, blood or brain cell, depending on the presence or absence of biochemical signals. Controlling this differentiation process is one of the biggest challenges in stem cell research.

Producing embryonic stem cell lines is tricky. Fewer than 150 lines have resulted from seven years of hard work.

There is nothing new about stem cells per se. Stem cell therapies have been used for decades. The best known example is bone marrow transplantation to treat leukaemia and other blood disorders; this works because marrow is full of blood stem cells. But all therapies so far have used what are often called adult stem cells—a term that is fine when the source is actually an adult but misleading when, as often happens, the cells come from an infant or foetus. Somatic stem cells may be a better name for these cells.

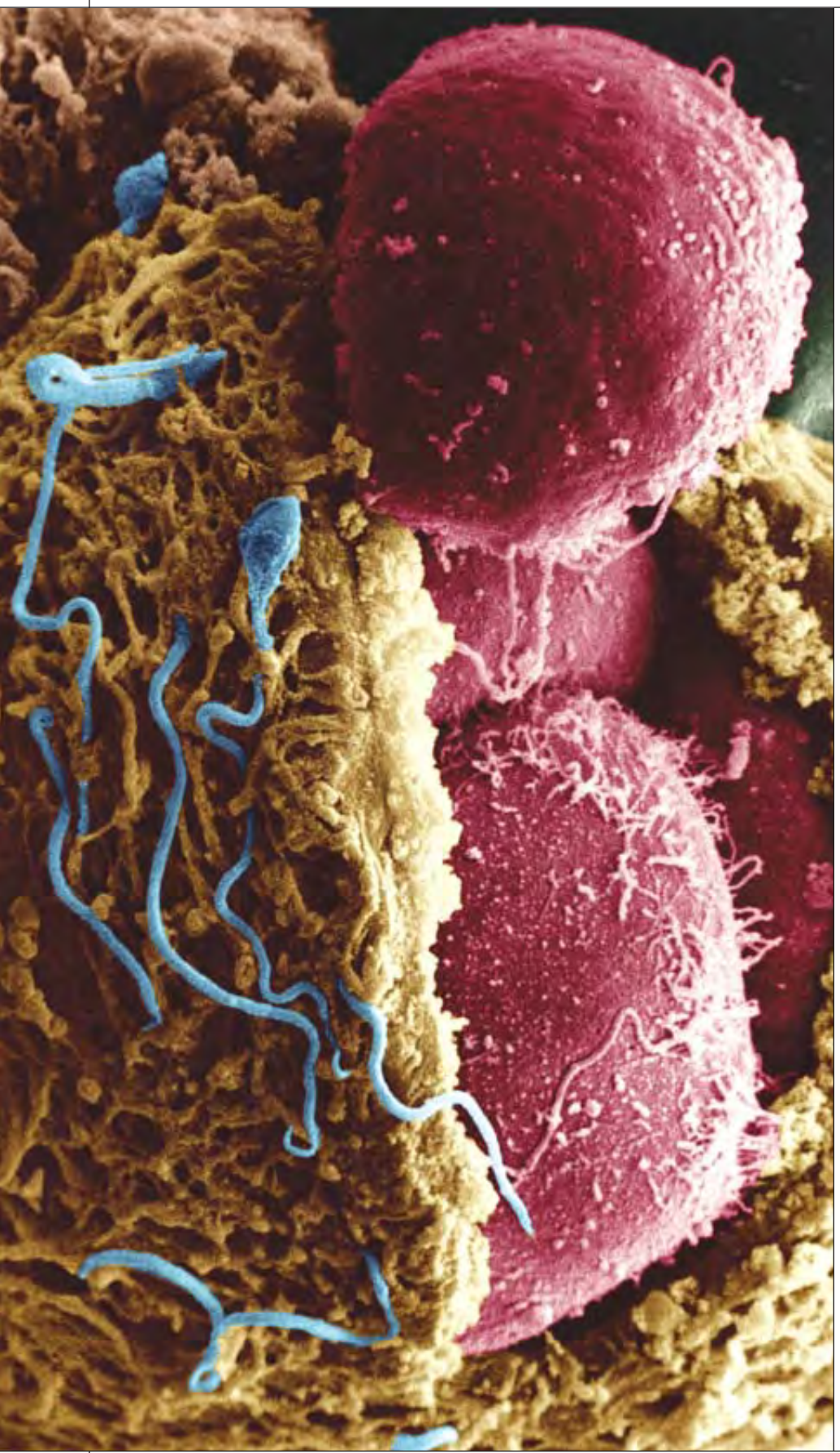
The range of specialised cells that can be obtained from somatic stem cells is limited—how limited is currently the subject of intense scientific debate that will be considered in a later article [see "Repair Workers Within," on page A12]. Early embryos are potentially a better source because all their cells are still unspecialised. Embryonic stem cells (commonly abbreviated to ES cells) are pluripotent: they can differentiate into almost any type of cell.

The first line (stable replicating population) of human ES cells was created in 1998 by James Thomson of the University of Wisconsin. The procedure involves taking cells from inside a week-old embryo (or blastocyst)—a microscopic ball of 50 to 100 cells—and culturing them in a laboratory dish with nutrients and growth factors. Embryos are normally donated by couples undergoing IVF treatment and would otherwise be discarded.

Even now, after seven years of intensive work worldwide, the world has fewer than 150 well-characterised ES cell lines, because the process of establishing them is extremely tricky. Only 22 lines are available for federally funded research in the US, where the Bush administration has decreed that the National Institutes of Health should not support work on lines created after August 2001. Once established, a stem cell line is essentially immortal. It can be frozen for storage in a cell bank, such as the one established last year in the UK, and for distribution to other researchers.

In an attempt to get round ethical objections to the destruction of human embryos for research, some scientists have been exploring alternative sources of ES cells. One approach would be to identify the least differentiated adult stem cells and wind back their developmental clock, so that they behaved as pluripotent ES cells. Another is through parthenogenesis—activating an unfertilised human egg so that

YORGOS NIKAS *Wellcome Photo Library*



PRECURSORS OF EMBRYONIC STEM CELLS (*red*) emerge from inside a four-day-old human embryo whose surrounding protein coat has been slit open. The cells can be harvested and cultured to give rise to embryonic stem cells.

it starts to divide like an early embryo. But it is not clear whether either approach will work in practice.

Until very recently, researchers have grown human ES cells on layers of mouse skin cells, known as feeder cells, which inhibit their differentiation into more specialised cells. They have also been nourished with blood serum derived from calf foetuses. Unfortunately, these nonhuman components carry a risk of contamination with animal proteins or pathogens, as in xenotransplantation, which could prevent the stem cells being used safely in the clinic.

This year several research groups have announced successful substitution of human for animal components, but some scientists maintain that contamination of the specialised media used for ES cell growth and differentiation is so pervasive that it will be hard to eliminate completely [*see box on page A11*].

ES cells, unlike adult stem cells, cannot be used directly in therapy because they cause cancer. Indeed, one laboratory test for ES cells is to inject them into mice and analyse the teratoma (a tumour formed of foetal tissue) that arises. So any therapeutic application will require scientists to drive the ES cells' differentiation into particular specialised cells for transplantation into patients—for instance, beta cells to produce insulin for diabetics or dopamine-producing neurones to treat Parkinson's disease. And rigorous screening will be required to make sure that no ES cells are still present.

If establishing ES cell lines is tricky, guiding their differentiation is a scientific nightmare. Researchers are only just beginning to understand the environmental conditions and the combinations of growth factors and other proteins required to guide human ES

Human-Animal Chimeras

Some experiments can disquietingly blur the line between species

Stem cell science has become notorious for obliging society to consider again where it draws the line between human embryonic cells and human beings. Less well known is that it also pushes us to another border that can be surprisingly vague: the one that separates people from animals. Stem cells facilitate the production of advanced interspecies chimeras—organisms that are a living quilt of human and animal cells. The ethical issues raised by the very existence of such creatures could become deeply troubling.

In Greek mythology, the chimera was a monster that combined the parts of a goat, a lion and a serpent. With such a namesake, laboratory-bred chimeras may sound like a bad idea born of pure scientific hubris. Yet they may be unavoidable if stem cells are ever to be realised as therapies. Researchers will need to study how stem cells behave and react to chemical cues inside the body. Unless they are to do those risky first experiments in humans, they will need the freedom to test in animals and thereby make chimeras.

Irving Weissman of Stanford University and his colleagues pioneered these chimera experiments in 1988 when they created mice with fully human immune systems for the study of AIDS. Later, the Stanford group and StemCells, Inc., which Weissman co-founded, also transplanted human stem cells into the brains of newborn mice as preliminary models for neural research. And working with foetal sheep, Esmail Zanjani of the University of Nevada at Reno has created adult animals with human cells integrated throughout their body.

The original chimera

MUSEO ARCHEOLOGICO, FLORENCE, ITALY/BRIDGEMAN ART LIBRARY

No one knows what the consequences will be as the proportion of human cells in an animal increases. Weissman and others, for example, have envisioned one day making a mouse with fully "humanised" brain tissue. The lawyer developmental programme and tiny size of this chimerical mouse fairly guarantee that its mental capacities would not differ greatly from those of normal mice. But what if human cells were instead put in the foetus of a chimpanzee? The birth of something less beastly could not be ruled out.

The intermingling of tissues could also make it easier for infectious animal diseases to move into humans. Diseases that hop species barriers can be particularly devastating because the immune systems of their new hosts are so unprepared for them (the flu pandemic of 1918 is widely believed to have sprung from an avian influenza virus).

There are currently no international standard governing chimera experiments. Canada's Assisted Human Reproduction Act of 2004 banned human-animal chimeras. The US has no formal restrictions, but Senator Sam Brownback of Kansas proposed legislation in March that would outlaw several kinds of chimeras, including ones with substantial human brain tissue. Some institutions that supply human stem cells set their own additional limits about what experiments are permissible.

Within the US, at least, greater uniformity may emerge from general guidelines on stem cell use recommended in late April by the National Academy of Sciences. The NAS recommended that chimeras involving most animal species generally be permitted. It urged a ban on any use of human cells in other primates, however, as well as the introduction of animal cells into human blastocysts. It also warned against allowing human-animal chimeras to breed: some human cells might have managed to infiltrate the animals' testes and ovaries. Breeding those animals could theoretically lead to the horrible (and in most cases, assuredly fatal) result of a human embryo growing inside an animal mother. —*John Rennie*

cells so that they become stable nerve or muscle or whatever other specialist cells are required for treatment.

Yet experience with mouse ES cells suggests that it will be possible to develop safe and effective therapies from their human counterparts. Researchers around the world are making a great effort to do so, because cell-based therapies are so immensely promising. Biologists believe most degenerative diseases are too complex to treat effectively just by giving patients drugs or even gene therapy. Living cells, which produce a far larger number of biologically active molecules, stand a better chance of success.

Although no clinical trials of ES cells have taken place yet, other types of cell therapy have shown that this kind of transplantation can work in people. Examples, besides the ubiquitous bone marrow transplant, include the use of neural stem cells from foetuses to treat brain disease and insulin-producing beta cells from cadavers to treat diabetes. Successes with somatic cells lie behind the hope that ES cells will eventually work even better, but a lot more research will be needed to prove the point.

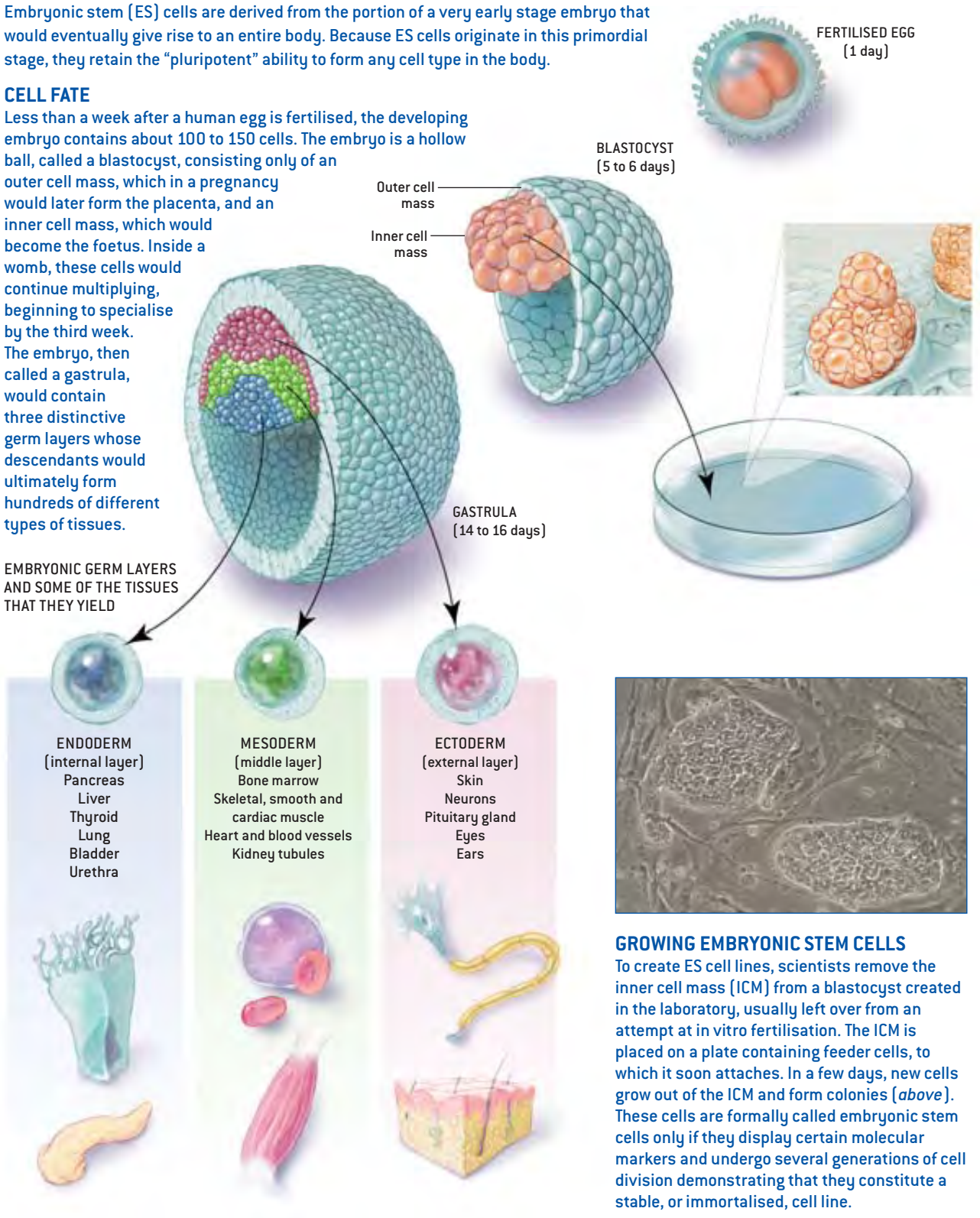
The obstacles that ES cell researchers need to overcome include better ways of obtaining ES cells efficiently; better methods to identify ES cells and their true developmental potential; ways to control their differentiation and growth inside the body; understanding whether the immune system attacks ES

The Origins and Fates of Embryonic Stem Cells

Embryonic stem (ES) cells are derived from the portion of a very early stage embryo that would eventually give rise to an entire body. Because ES cells originate in this primordial stage, they retain the "pluripotent" ability to form any cell type in the body.

CELL FATE

Less than a week after a human egg is fertilised, the developing embryo contains about 100 to 150 cells. The embryo is a hollow ball, called a blastocyst, consisting only of an outer cell mass, which in a pregnancy would later form the placenta, and an inner cell mass, which would become the foetus. Inside a womb, these cells would continue multiplying, beginning to specialise by the third week. The embryo, then called a gastrula, would contain three distinctive germ layers whose descendants would ultimately form hundreds of different types of tissues.



GROWING EMBRYONIC STEM CELLS

To create ES cell lines, scientists remove the inner cell mass (ICM) from a blastocyst created in the laboratory, usually left over from an attempt at in vitro fertilisation. The ICM is placed on a plate containing feeder cells, to which it soon attaches. In a few days, new cells grow out of the ICM and form colonies (*above*). These cells are formally called embryonic stem cells only if they display certain molecular markers and undergo several generations of cell division demonstrating that they constitute a stable, or immortalised, cell line.

ANDREW SWIFT; ROBERT P. LANZA *ACT* (*micrograph*)

Dirty and Dying, but US-Approved?

Problems with contamination and genetic abnormalities may not stop work on embryonic stem cell therapies

In August 2001 when President Bush forbade the creation of new embryonic stem cell lines with federal money, he softened the blow to biomedical research by promising that more than 60 ES cell preparations could still be used to develop prospective treatments for the sick. Yet a growing list of problems with those cells forces the Food and Drug Administration to consider whether material from them is even safe to try in people.

Only 22 of the sanctioned ES cell lines created before August 2001 have survived and remain available to researchers, although questions have arisen about their quality in light of their advancing age. The lines are supposed to be "immortal," but being kept in culture for extended periods has been known to induce deformities in other cells, so scientists were not entirely surprised when reports emerged of major genetic abnormalities in some of the National Institutes of Health registry lines. Other registry cell lines simply seemed to lose their ability to produce differentiated cell types or would only do so sluggishly.

HUMAN EMBRYONIC CELLS grown in the laboratory have been contaminated with material from supportive mouse cells in the cultures, which makes their usefulness in future therapies questionable.

Methods for handling stem cells have improved considerably since the US policy went into effect, and researchers believe that fresher cell lines can be kept much healthier. In particular, two new types of culture medium unveiled this year eliminate the need to grow ES cells on beds of mouse "feeder" cells, a practice used on all the government-approved lines in the past. Fears that the registry cells might have been contaminated with mouse molecules were recently confirmed by a study showing that human ES cells grown in this way absorb a mouse protein and carry it on their surface. When ES cells displaying the protein were exposed to human blood serum, antibodies against the animal protein attacked and killed the ES cells.

Nonetheless, California-based Geron, which owns rights to nine of the government-approved lines, says it will apply to the FDA early in 2006 for permission to go ahead with human trials of the cells for spinal repair. Thomas Okarma, Geron president, is confident the company's cells are clean after subjecting them to what he calls an "exhaustive list" of "gold standard" tests. No other US company has announced a formal application to try embryonic stem cell derivatives in people, but a director of the University of Minnesota's Stem Cell Institute, John Wagner, reportedly told state legislators last year his group had already sought FDA approval for such a trial. Wagner declined to reveal any more details.

Nor will the FDA comment on how many applications it has received for trials of ES cell derivatives or when it will rule. The possibility of animal contamination does not automatically preclude use of registry cells in humans—xenotransplantation of pig heart valves and even a baboon-to-human bone marrow transplant have gained FDA approval in the past. The only remark a spokesperson would offer was that the agency's decision will be based on the scientific soundness of the proposed trial, not politics.

—Christine Soares

cells or ones differentiated from them; and learning more about the comparative advantages of ES cells and somatic cells for various applications.

While direct use of stem cells in patients is what most excites politicians and the public, many scientists say their main medical benefits may be delivered indirectly, through their use in research to advance other therapies. If researchers can work out the complex chemical and genetic signals that control the growth and differentiation of stem cells, the results would be enormously useful in medicine. ES cells should make it possible to develop models of tissue development and function that will enable chemists to test potential drugs more effectively.

For example, if ES cells derived from embryos known by genetic screening to carry cystic fibrosis genes can be guided to become CF lung cells, these would open a new window for studying the disease and testing treatments for it. For pharmaceutical chemists, unlike biologists, the vision of regenerative medicine involves finding drugs—ideally small molecules that patients can take by mouth to stimulate their own tissues to regenerate—rather than messing around with cell therapy.

The science is still far too uncertain for us to tell how stem cell research and regenerative medicine will develop. It may take another generation or two before we derive much clinical benefit from the great biological advances of the late 1990s. But the medical payoff could eventually be spectacular.

GETTY IMAGES

The Cloning Connection

Cloned tissues from stem cells might beat immune rejection

Stem cell scientists are often irritated by the way people confuse their work with cloning, even though cloning plays no part in most ES cell research today. One reason for confusion is simply that both fields involve creating embryos.

Another may be an accident of timing: human ES cells were first cultured soon after the birth of Dolly, and commentators immediately pointed out the potential for combining the two discoveries. The term "therapeutic cloning" was coined to describe the creation of a cloned embryo as a source of ES cells; the embryo is destroyed in the process. In contrast, reproductive cloning would produce a baby from the cloned embryo.

Yet there is no denying that cloning is an important item on the stem cell research agenda, because it seems the best way to overcome a serious clinical problem with cell and organ transplantation: immune rejection. The immune system attacks any graft that is not genetically identical to the patient. Even a well-matched transplant requires lifelong treatment with immunosuppressive drugs, which have serious side effects, including increased susceptibility to infection and cancer.

Therapeutic cloning uses somatic cell nuclear transfer (SCNT), the technique that gave rise to Dolly: the nucleus of one of the patient's cells is transferred into a donated egg whose own nucleus has been removed. The egg is then stimulated to behave as if it has been fertilised, developing into an embryo that could be a source of ES cells with the same DNA as the patient. (Opponents of cloning point out that the same embryo could be implanted into a womb and grow into a baby.)

Unfortunately, SCNT is an inefficient process, in animals and people. The first scientifically credible account of human cloning came last year from Woo Suk Hwang and his colleagues at Seoul National University; they used 242 eggs to obtain 30 early embryos, from which they derived just one viable line of ES cells. South Korea has a culture of egg donation for research, which enabled the scientists to obtain good-quality eggs.

Indeed, even if therapeutic cloning can be made efficient, it is hard to see how enough human eggs could be made available to use the procedure in the clinic on a large scale (unless there is an unforeseen technical breakthrough).

In the more immediate future, however, scientists hope to use therapeutic cloning as a research tool that could give new insights into disease. While genetic disorders such as cystic fibrosis can be studied by deriving ES cells from embryos known to carry the single defective gene in question (*see main article*), this is not possible for diseases that result from multiple or unknown factors.

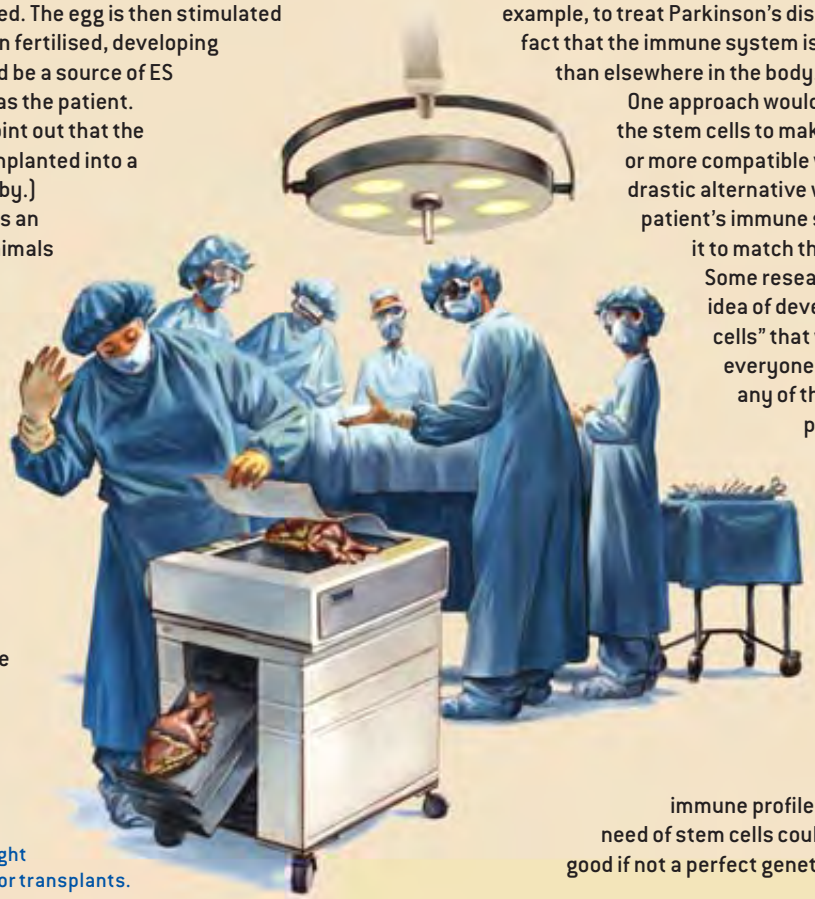
Last Month Hwang's group in Korea announced the derivation of ES cell lines cloned from a range of patients suffering from inherited diseases or spinal cord injury. The efficiency of the process has improved, too: 185 donated human eggs yielded 31 cloned embryos and 11 ES cell lines. Lab tests confirmed that each cell line was immunologically compatible with the patient from whom it was derived.

Meanwhile other researchers are looking for alternative approaches to reducing immune rejection of stem cells. Some say even that the whole issue may have been exaggerated, because embryonic and foetal cells are intrinsically less immunogenic than adult cells—and they point out that neural transplants, for example, to treat Parkinson's disease, will benefit from the fact that the immune system is less active in the brain than elsewhere in the body.

One approach would be somehow to engineer the stem cells to make them less immunogenic or more compatible with the patient. A more drastic alternative would be to wipe out the patient's immune system and reconstruct it to match the transplanted cells.

Some researchers have floated the idea of developing "universal donor cells" that would be compatible with everyone. But it is not clear whether any of these methods would work in practice.

Perhaps more achievable, though still an ambitious long-term project, is the idea of minimising rejection, rather than avoiding it altogether, by building up stem cell banks with many hundreds or thousands of cell lines representing as complete a spectrum of immune profiles as possible. Any patient in need of stem cells could then expect to receive a good if not a perfect genetic match. *—C.C.*



THERAPEUTIC CLONING might duplicate organs needed for transplants.

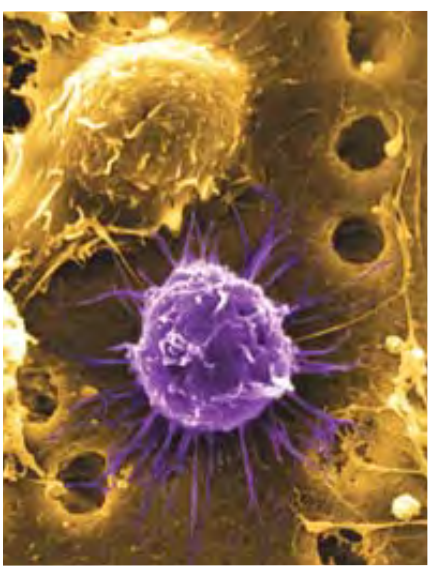
MATT COLLINS

Repair Workers Within

Adult stem cells may escape the ethical controversies of their embryonic counterparts, but as Christine Soares notes, their practical clinical value is far more murky

Using stem cells for clinical therapies is an idea still bathed in a futuristic glow, but one such treatment already has a history of success going back almost 40 years. Tens of thousands of patients treated with bone marrow transplants have shown that an infusion of healthy stem cells can regenerate a failing body part. In most of these cases, the patients suffered from congenital blood or immune disorders, or their bone marrow had been damaged by cancer treatment. As a result, the haematopoietic stem cells in their marrow, which normally produce billions of blood and immune cells daily, needed replacing.

Since 1968, these transplants have triumphantly repaired patients' capacity to manufacture healthy blood and immune cells. Over the past decade, as scientists discovered additional stem cell types throughout the human body, enthusiasm has grown for the possibility that other failing body parts might also be regenerated with a transplant of stem cells.



HAEMATOPOIETIC STEM CELL (*purple*) is derived from bone marrow. This was the first type of adult stem cell used therapeutically to regenerate blood and immune cells via bone marrow transplants.

Yet the more researchers learn about the characteristics and behaviour of adult stem cells, the less they seem to agree on answers to some fairly fundamental questions, such as what these cells really are, where they originate, what they are capable of doing, and how they do it. Consequently, although adult stem cells may not provoke much political rancour today, they have become more scientifically controversial than their embryonic counterparts.

Fortunately, the majority of scientists can at least agree on a basic definition: a stem cell (whether adult or embryonic) must renew itself indefinitely through cell division, while remaining in its generic state and retaining its potential to give rise to daughter cells of more specialised types. These progeny often start out only partially differentiated themselves, with some flexibility to serve as progenitors of several cell varieties within a particular organ or system [*see box on opposite page*]. For example, descendants of mesenchymal stem cells found in bone marrow can become bone, as well as cartilage, fat cells, various kinds of muscle and the cells that line blood vessels.

Although the tissues that sprout from these bone marrow stem cells are seemingly diverse, they have one thing in common: when the human body is first forming, they all originate in the middle layer, or mesoderm, of the developing embryo. This fact is at the heart of one of the most important questions debated by stem cell scientists: whether adult stem cells can transdifferentiate, that is, produce functional new tissues outside the lineage of their embryonic layer. The answer could be crucial to some of the more ambitious regenerative therapies based on adult stem cells.

Traditionally, adult stem cells have been considered limited in their potential, able only to manufacture cell varieties within their own lineage. Hence, they are usually described as multipotent, rather than pluripotent like embryonic stem cells. In recent years, however, many research groups have claimed to have made adult stem cells cross lineage lines—for example, by turning haematopoietic stem cells into liver, neural stem cells into blood vessels and mesenchymal stem cells into neurones.

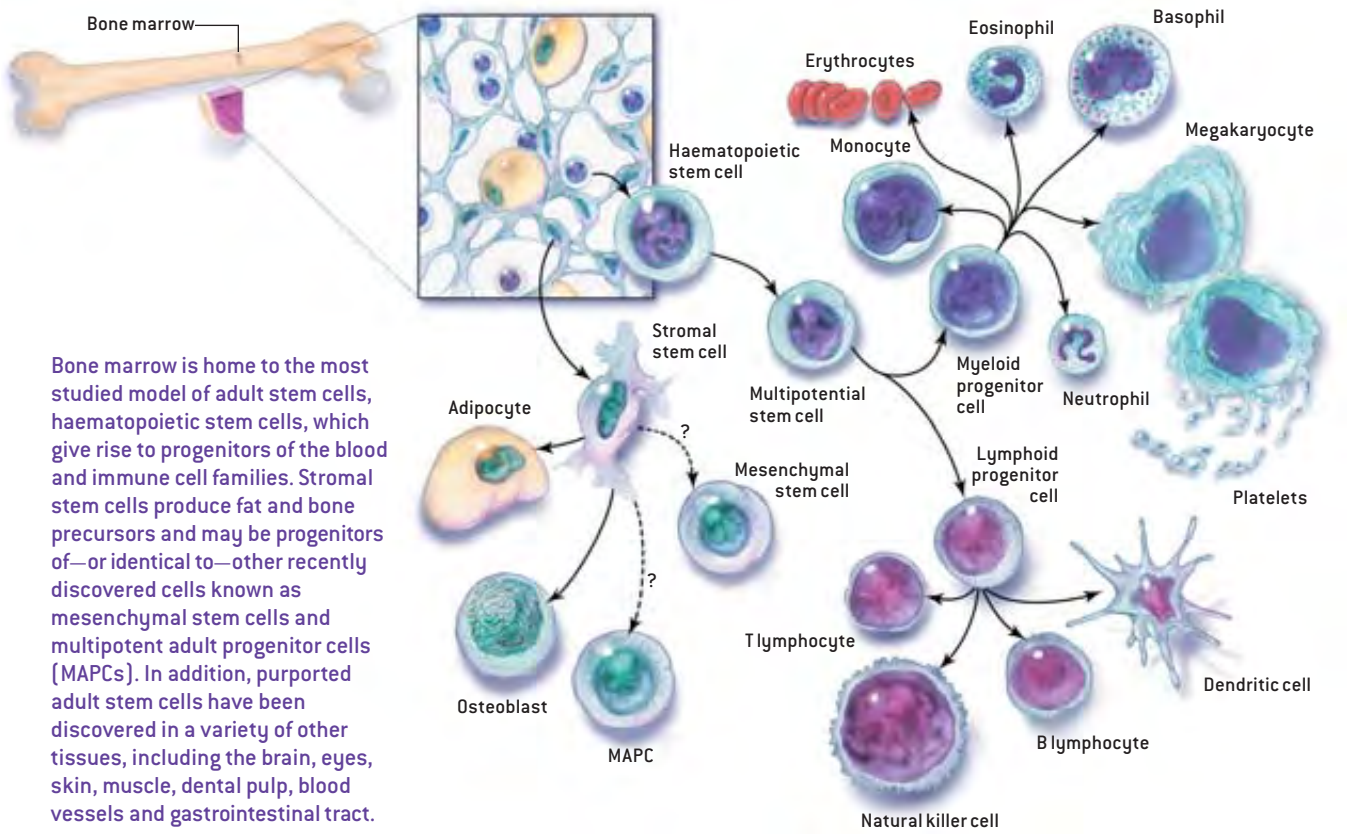
In 2002 Catherine Verfaillie of the University of Minnesota first described a new adult stem cell from bone marrow that could produce cell types of all three embryonic lineages. Dubbing it a multipotent adult progenitor cell (MAPC), Verfaillie speculated that its flexibility might equal that of embryonic stem cells. Indeed, she thought MAPCs might be left over from embryonic development to serve as a universal repair mechanism for the adult body.

Such a one-size-fits-all adult stem cell would certainly solve the problem of regenerating tissues where no local progenitors have been discovered, such as in the adult heart, or where local stem cells are extremely rare and difficult to obtain, as in the brain. Unfortunately, other investigators have had difficulty reproducing some of the original MAPC results, so the jury is still out on their real potential. Further scrutiny has also thrown cold water on many of the transdifferentiation claims for other types of adult stem cells.

Even in tissues that share a lineage, transplanted stem cells do not always work enthusiastically. In particular, at-

ANDREW LEONARD *Photo Researchers, Inc.*

Stem Cell Storehouse



Bone marrow is home to the most studied model of adult stem cells, haematopoietic stem cells, which give rise to progenitors of the blood and immune cell families. Stromal stem cells produce fat and bone precursors and may be progenitors of—or identical to—other recently discovered cells known as mesenchymal stem cells and multipotent adult progenitor cells (MAPCs). In addition, purported adult stem cells have been discovered in a variety of other tissues, including the brain, eyes, skin, muscle, dental pulp, blood vessels and gastrointestinal tract.

tempts to make stem cells taken from blood or bone marrow generate new tissue in the heart have produced conflicting results.

In clinical trials involving patients whose hearts were scarred by heart attacks, modest tissue regeneration has sometimes been observed. This improvement can occur even when the studies find no evidence that the stem cells contributed new heart cells to the healing organ. The key to this seeming paradox may be that stem cells can secrete helpful growth-signalling chemicals and contribute to the formation of new blood vessels. In other words, the transplanted bone marrow stem cells may not be producing new heart cells themselves, but they could be laying essential groundwork for the heart's own as yet undiscovered progenitor cells to do so.

Opponents of further human testing have argued that performing these transplants before the regenerative mechanisms at work are fully understood puts patients unnecessarily at risk for tumourlike growths or abnormal heartbeats. Given the lack of effective alternatives for people with failing hearts, however, the trials are very likely to continue, making heart repair potentially the first widespread therapeutic application of adult stem cell therapy beyond traditional bone marrow transplants.

Treatments for less life-threatening conditions may not be far behind. An ongoing clinical trial is already testing the safety of breast reconstruction material created from the stem cells found in fat. In the past two years, both skin and hair stem cells have also been discovered, each of which might be marshalled for cosmetic work. Dental researchers hope to make stem cells discovered in and around teeth regenerate enamel or crowns, although growing an entirely new tooth from scratch might be more than adult stem cells could muster anytime soon.

So far the cells seem to do best when applied within their own lineage to produce small amounts of new tissue or to boost natural regeneration. Last December, for example, German doctors reported having repaired a large gap in a young girl's skull using a combination of bone graft and stem cells derived from her own fatty tissue.

Injections of fat-derived stem cells are already gaining popularity as a means to speed healing of bone and cartilage injuries in horses. For certain uses in humans, too, these cells could be easier to harvest than mesenchymal stem cells from bone marrow. Researchers are finding, however, that like all other adult stem cells studied to date, this type shows a definite decline in vi-

ANDREW SWIFT

Patient, Heal Thyself

Revving up the body's own stem cells could be the simplest route to new therapies

The body's innate capacity for regeneration is what all stem cell therapies strive to emulate and improve upon. For that reason, the simplest route to many treatments may involve recruiting and activating the stem cells already hiding within our tissues. A major medical research effort now focuses on learning the subtle chemical language that directs stem cell behaviour during natural wound healing. Mastering this idiom could in some cases help to eliminate the need for therapeutic infusions of lab-grown cells. The right chemical cues might even restore the vigour to cells in older patients. The potential benefits are many—but there are also dangers.

To see the benefits, consider the aftermath of an overzealous workout that leaves muscles screaming in pain. Individual muscle cells release chemical signals as their own cry for help. Homing to the sites of microscopic tears in the muscle fibres, the stem cells then immediately get to work making repairs.

Early this year a newly discovered protein dubbed Delta was credited with rejuvenating the muscle-building stem cells of mice. A group led by Stanford University's Thomas Rando paired old and young mice, connecting their circulatory systems so that the old mice had the youngsters' blood running through their veins. Rando found that something in the young blood, purportedly the Delta protein, restored youthful activity levels to stem cells belonging to the old mice.

Researchers have in the past successfully regenerated muscle mass in animals through experimental gene therapies that deliver a different protein, called insulinlike growth factor-1 (IGF-1). (Indeed, the experiments worked so well they have triggered fears that future athletes will engage in "gene doping".) IGF-1 both triggers stem cell activity and, when its call is amplified, can summon stem cells from afar to the site of an injury. Rather than requiring transplanted stem cells to regenerate tissue damaged by a heart attack, therefore, some researchers believe a dose of IGF-1 could kick-start repairs by stem cells already circulating in the bloodstream or hiding within the heart itself. A similar approach might work in any number of organs or tissues, provided scientists can learn which signals call the correct stem cells to duty.

But even more important may be knowing how to shut the stem cells off when the repairs are done. One of the darker revelations to have come from stem cell research in recent years is the connection to some varieties of cancer. At least one leukaemia is known to be caused by bone marrow stem cells gone awry. Certain brain, stomach and breast cancers are also now suspected to be triggered by stem cells turned malignant.

One theory holds that this may happen when stem cells, which are usually dormant, get stuck in wound-repair mode. Remaining activated too long makes the stem cells vulnerable to genetic mutations, and then they can become a biological nightmare: a rogue cancer cell with a stem cell's proliferation power.

Yet researchers are already finding ways to turn the stem/cancer cell connection back to patients' advantage. The homing instinct of stem cells has been exploited in animal experiments to deliver a "suicide gene" to tumour cells, leaving normal tissues unharmed. The physical similarities of cancer and stem cells also recently led to a mechanical test that makes it easier to find both types of cell in a person's blood. And, of course, widespread attempts to parse the signalling language of stem cells in order to turn a patient's own healing powers on may also reveal commands that turn tumour cells off. *—C.S.*

MATT COLLINS

gour as their owners age. Late in life when repairs are most likely to be needed, one's own stem cells might therefore not be the best bet. Where, then, might patients turn?

One potential source of fresh therapeutic stem cells is the donated tissue of miscarried and aborted foetuses. These stem cells are classified as "adult" because they are found in differentiated tissues. Their extreme youth, however, gives scientists hope that when transplanted they will adapt easily to new surroundings and energetically produce new cells.

A major test for both foetal stem cells and the prospects of cell-based brain therapies in general could come in the next year if California-based StemCells, Inc., receives US government approval for its proposed clinical trial. The company, co-founded by the Salk Institute's Fred Gage, who first discovered neural stem cells, plans to transplant foetal neural stem cells into the brains of children with Batten disease. That lethal illness arises from the failure of brain cells to produce an enzyme that clears away cellular wastes. If the stem cells manufacture healthy new brain cells that produce the missing enzyme, the treatment could alleviate the disease, with exciting implica-

tions for other related brain disorders.

The Batten trial would be Western scientists' first transplant of neural stem cells into the human brain, an environment that some fear could be difficult for stem cell therapy. Unlike skin, liver and other tissues that naturally repair themselves after an injury, the brain, spinal cord and other nervous tissues do not, and no one is quite sure why. The very existence of adult neural stem cells suggests that they should be able to replace damaged neural tissue. Their failure to do so has prompted speculation that something inhibits them.

Researchers at the Schepens Eye Research Institute in Boston, Mass., reported a breakthrough on this problem earlier this year. Just by manipulating genes responsible for sending "blocking" signals to stem cells, they were able to regrow damaged optic nerves in mice. The experiment highlights a new and promising approach to stem cell therapy. The idea is to learn the language of signals that normally direct stem cells' behaviour well enough to be able to recruit a patient's own stem cells to make repairs on demand [*see box on opposite page*].

Studying the cues that stem cells send and receive in their natural environment is also improving scientists' basic understanding of what gives a stem cell its potential. If the secret to "stemness" were as simple as having particular genes active at specific times, then any cell of the body might conceivably be turned into a stem cell as needed [*see box at right*].

Ongoing investigations of both adult and embryonic stem cells will likely reveal whether such a feat is feasible. The adult versions so far appear to lack the versatility of the embryonic kind, and even within their own tissue families they show diminishing vigour. Still, certain types of adult stem cells have already proved themselves extremely useful for modest regeneration and repairs. The diverse research currently focused on these cells worldwide promises to unlock further the power of the body's own repair system.

ANDREW SWIFT

Making Stem Cells on Demand

Changing muscle into bone and regrowing organs could be the fruits of work on "dedifferentiation"

What can a simple newt do that humans are trying to learn? The tiny amphibian can regenerate an entire lopped-off limb, or a whole organ, by taking normal, differentiated body cells—bone, skin, muscle and so on—and winding back their clocks to an undifferentiated state of stemness. Newts create these instant stem cells at the site of an injury, then immediately begin rebuilding the missing body part.

In contrast, once a mammal's cells have gone down the path of becoming bone or skin or brain cells, there is normally no turning back. They are said to be terminally differentiated. If humans could undo differentiation, though, doctors might not have to hunt for rare and elusive stem cells within the body or try to force stem cells from one tissue to regenerate tissue of another type. Instead an ordinary pancreas cell might be turned into a progenitor of the insulin-producing cells lost in Type 1 diabetes. Normal nerve cells could become a neurone factory for brain or spinal cord repair.

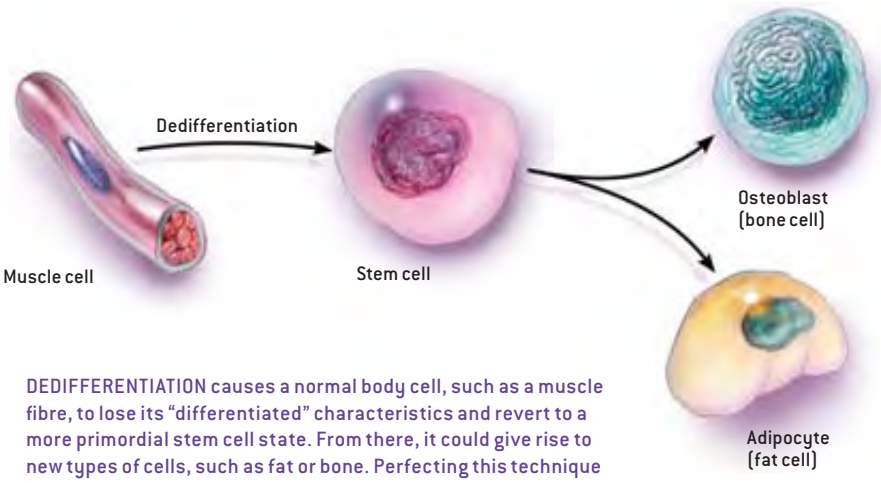
Investigations of this approach are just beginning, but early results are both encouraging and intriguing. Harvard Medical School's Mark Keating and his colleagues first showed in 2001 that dedifferentiation in mammals might be possible by regressing mouse muscle cells with an extract from regenerating newt limbs. They attributed the reversion to proteins in the extract having switched on one or more genes in the cells.

Last year a group from the Scripps Research Institute also reported dedifferentiating mouse muscle and then turning the cells into bone or fat. They used a small-molecule chemical that they found by trial and error and have named reversine, but as yet they are not sure how it worked.

Others are studying the natural environments, or niches, that stem cells usually inhabit within the body to figure out which environmental cues may tell stem cells what to do and when to do it. Allan Spradling and Toshie Kai of the Carnegie Institution of Washington have used this kind of information to control fruit-fly stem cells that normally produce the female's eggs. By manipulating niche signals, they could make the stem cells differentiate, then dedifferentiate again.

These kinds of results fuel speculation that such environmental signals may be crucial to creating and maintaining the stemness of stem cells. As Dov Zipori of the Weizmann Institute of Science in Rehovot, Israel, put it in a recent review article, a stem cell may turn out to be not an entity so much as a state—one that any cell could enter under the right conditions.

—C.S.



DEDIFFERENTIATION causes a normal body cell, such as a muscle fibre, to lose its "differentiated" characteristics and revert to a more primordial stem cell state. From there, it could give rise to new types of cells, such as fat or bone. Perfecting this technique would mean that regular body cells might be turned into an unlimited supply of stem cells for tissue regeneration.

A Patchwork of Laws

Richard Gardner and Tim Watson find much disagreement around the world about what should be allowed with stem cells—in spite of attempts at finding consensus

Whether scientists can capitalise on the huge potential that stem cell research and therapeutic cloning promise depends on where in the world they work. There is a disparate and confusing patchwork of legislation, with little agreement between countries on exactly what should be permitted and what should be banned. Attempts to reach consensus have failed in Europe and at the United Nations, and in some countries the debate remains unresolved at the national level.

The science is complex, and the ethical dimensions equally so. But the problem lies in the major differences of opinion over which parts of the science are considered acceptable.

There are three main scientific issues at the heart of the debate—human embryonic stem cells, reproductive cloning and therapeutic cloning. To some, all three are equally unacceptable, but to others they are different enough to merit separate consideration.

The source of human embryonic stem cells is a major point of contention, as they are taken from embryos that are just a few days old. They are primarily taken from embryos that have been left over from fertility treatments, but this limits the types of research that can be carried out. A possible alternative, and one that raises further moral quandaries, is to produce cloned embryos.

Since the cloning of Dolly the sheep in 1997, the world has had to grapple with the serious prospect that cloning a human might indeed be possible. The single point which all countries seem agreed upon is that, for now, attempting to create a human clone, also known as reproductive cloning, is scientifically unsafe, ethically unsound and unacceptable socially.

But there is a related procedure known as therapeutic cloning whereby the early embryo never develops beyond a microscopic ball of cells in the laboratory. During this time, research is carried out on it, most often to extract stem cells, but it can also be to understand better the early development of genetically based diseases.

Some countries have put in place total bans on all forms of human cloning, others have banned reproductive cloning but still allow therapeutic cloning and some have so far failed to introduce any regulations, often as the result of a failure to reach agreement. Many countries also have regulations on the derivation and use in research of human embryonic stem cells.

To illustrate the range of regulation, we can look at the huge differences between the US and the UK.

The UK is one of a handful of countries to have introduced legislation with the express purpose of allowing the use of human embryos for stem cell research and therapeutic cloning. In 2001 the UK introduced primary legislation against reproductive cloning; however, this action was taken after it had extended the terms of the Human Fertility and Em-

BRAZIL'S SCIENCE AND TECHNOLOGY MINISTER Eduardo Campos (*at far left in back row*) celebrates with handicapped people the passage of a stem cells law on March 2, 2005. Their T-shirts trumpet, in Portuguese, the *esperança*, or hope, that people from all over the world hold for therapies that may come from *células-tronco*, or stem cells.

ROSE BRASIL *AP Photo*

The Next Frontier: The Courtroom

As arguments mount over who will own the future technologies born of stem cell research, corporate lawyers prepare for battle

Who owns stem cells? And more to the point, who should own the life-altering medical treatments that may one day emerge from this futuristic and highly contentious field of research?

It may seem premature to worry about ownership rights for technologies that do not yet exist—and may never prove commercially viable. But with more money pouring into embryonic stem cell research—especially after the success of a ballot initiative in California last year, mandating $3bn in state funding for embryonic stem cells—disputes over ownership rights cannot be far behind, legal experts say.

There has been very little US litigation over stem cells. The truce may not last.

Stem cell research has been a focus for intense political and ethical battles for years. Now the next frontier is in the courts: battles over who owns what in a field where intellectual-property rights are far from clear.

"Typically litigation only arises when there are commercially available products and a very real market for the technology", notes Bill Warren, an expert on biotechnology patents at the law firm Sutherland Asbill & Brennan in Atlanta. But now that California and other states are getting into the game of financing stem cell research, that will hasten the development of the technology, says Warren, and "litigation will definitely be coming", possibly in the next five years.

Up to now, legal experts point out, there has been very little US litigation involving stem cells, even though one organisation claims to own the patent rights to all embryonic stem cells. That group, the Wisconsin Alumni Research Foundation (WARF), says its patents cover "a method of culturing human embryonic stem cells and composition of matter which covers any cells with the characteristics of stem cells"—in other words, pretty much anything to do with embryonic stem cell research.

Critics, in the academic and commercial research communities, complain that this patent is too broad. But WARF and the US Patent and Trademark Office defend it, on the grounds that if others believe they have rival rights, they can fight it out in court.

And despite the breadth of its patents, WARF is so far not impeding anyone else's research activities, says Arti Rai, an expert on scientific patents at Duke University Law School, pointing out that WARF freely licenses its patent for research purposes. But the current truce may not last long, she states, once WARF's rivals in the field are ready to commercialise their own technology. At that point, the breadth and validity of WARF's patents will be challenged in court.

Critics who see stem cell patents as an impediment to the development of lifesaving technologies are just plain wrong, says Michael Werner, chief of policy at BIO, the Biotechnology Industry Organisation. "Intellectual property is critical to scientific advancement", he observes. "There would be no private investment without patent rights". The only thing that will stifle stem cell research, he adds, is threatening the IP rights of those who carry it out for profit.

He places the debate over stem cell patents squarely at the centre of a larger social debate—in the US and elsewhere—over how to balance the intellectual-property protection needed to convince companies to invest in innovation with the need to maintain the kind of vibrant public domain that also is capable of fostering progress.

Everybody knows somebody who could one day be helped by a medical treatment based on stem cell technology. But the legal questions surrounding this promising technology are almost all as yet unresolved. And the issue of who owns the results of stem cell research can only get more complicated, as more and more American states start their own programmes to fund stem cell experimentation, creating a tangled web of private and public financing that can only, in the end, be resolved by the courts.

—Patti Waldmeir

bryology Act governing licensed research on early human embryos.

These measures were taken following wide public debate and were passed by majorities of more than two to one in both Houses of Parliament. The Royal Society, as the UK's national academy of science, played a significant role in informing the debate during this process. The result has been a carefully regulated process, which has so far resulted in two licences being granted to carry out research into diabetes and into motor neurone disease.

By stark contrast, in the US, despite an influential religious lobby consistently condemning any research involving embryos, there is no primary federal legislation to regulate any form of human cloning. This reflects a split between those who strongly believe all cloning should be banned and those who wish to see only reproductive cloning banned and an inability to come up with suitable legislation, despite numerous and ongoing efforts.

The latest development was the resubmission of the Human Cloning Prohibition Act of 2005 to Congress by Senator Sam Brownback of Kansas on March 17. This proposed a federal ban, which makes no distinction between reproductive and therapeutic cloning and has strong support but has already failed to make it into law twice since 2001. Brownback has also declared his equally strong opposition to any effort in the House of Representatives to reconsider an existing ban on

Engineering Aside the Morality

Researchers ponder how to procure ES cells without destroying embryos

What if science, with a shake of a test tube, could circumvent the ethical objections to embryonic stem cell research? Several proposals would in principle let scientists obtain precious embryonic stem cells without harming embryos (equally precious to some) in the process. For eager biotechnologists, that arrangement would sound almost too good to be true—and indeed, it most likely is.

William B. Hurlbut of Stanford University, a member of the US President's Council on Bioethics who is a firm believer in the "implicit moral dignity" of the embryo, has attracted attention by suggesting a combination of genetic engineering and cloning called altered nuclear transfer. In one scheme, the nucleus of a mature cell would be extracted and altered to turn off one or more genes that are vital during an embryo's development. The nucleus would be injected into a prepared egg cell that is then zapped with electricity to activate it, as in cloning. If all goes as it should, this biological entity, which Hurlbut says "never rises to the level of what can properly be called a living being", would become at most an unorganised clump of embryonic cells suitable for scientific research and possibly clinical treatments.

> Production of what amounts to sacrificial monsters is unlikely to satisfy those who believe that any tinkering with the primordial stuff of life is wrong.

Not all bioethicists share Hurlbut's enthusiasm for this plan. That cellular clump would bear a great likeness to a teratoma—a grotesque tumour mixing together different cell types, from hair to muscle to teeth. Although it may not be classifiable as an embryo, in the eyes of many, it certainly triggers what Leon Kass, the chairman of the council, has called the "yuck factor" for viscerally identifying unethical practices. Critics have also questioned whether intentionally creating a doomed abomination is morally superior to destroying embryos that already have no future. And yuckiness aside, to make successfully even one line of stem cells in this way, hundreds of human eggs might be needed, which itself entails ethical and technical problems.

Two Columbia University researchers have circulated a perhaps more pragmatic idea: pluck living ES cells from the many embryos produced in vitro that have died spontaneously. Donald W. Landry and Howard A. Zucker have begun work on tests for assessing markers such as the final arrest of cell division, which the scientists equate with "brain death" for embryos.

Ironically, the Landry/Zucker scheme would rescue nominally healthy cells from dead embryos, while healthy but unused IVF embryos would continue to be discarded. It also forgoes the dream of someday cloning ES cells from a patient's own body for use in treatments. Such bespoke stem cells would be safe from immune rejection; ones derived from dead embryos would not be. Hundreds of thousands of cell lines might therefore need to be cultured and stored to provide all patients with immunologically compatible cells.

Other would-be solutions include techniques for extracting individual stem cells without harming embryos and for using unfertilised human eggs coaxed into a short-lived process resembling embryo formation. Another straightforward strategy would avoid ever going near an embryo. Instead an adult stem cell would be forced to "dedifferentiate", or revert to its more embryonic pluripotent state. At the moment, however, such a concept borders more on alchemy than biochemistry. A US National Academy of Sciences report issued in April summarised these approaches as seeming to have numerous technical hurdles for now.

A critique in the *New England Journal of Medicine* specifically aimed at Hurlbut's proposal may further dampen all these ideas. Douglas Melton, George Daley and Charles Jennings of Harvard University argued that the switching off of a gene does not represent "a transition point at which a human embryo acquires moral status". No similar developmental or biochemical benchmark may ever lend ethical certitude to this field. Industrial-scale production of sacrificial monsters is unlikely to satisfy those who believe that any tinkering with the primordial stuff of life is wrong.

—Gary Stix

federal funding of some embryonic stem cell research.

Worryingly, no federal legislation exists to stop a privately funded laboratory attempting to create a human clone. But any outcome of research would then be subject to Food and Drug Administration approval, which it would be extremely unlikely to pass.

Scientists can receive federal funds to use human embryonic stem cells in their research, but only the cell lines created prior to 2001, of which only 22 are available. Also, some states have now enacted their own legislation, in some cases to ban all cloning and embryonic stem cell research and in others to allow therapeutic cloning and even pledge millions of dollars of funding, most notably in California.

Countries where therapeutic cloning and stem cell research are permitted often regard it as great news that the US is lagging behind. Levels of investment in this kind of research in the UK are testament to this. But in the long term, losing out on the expertise and resources of the world's leading scientific nation means patients around the world will lose out, too, because a global effort is needed to make the most rapid progress.

Elsewhere, the opinions and legislation are equally varied. Europe is

divided on the issues. Most countries, including Germany, Austria, France and the Netherlands, have brought in legislation to ban reproductive and therapeutic cloning. Yet they are in the curious position of not going as far as countries such as Italy, Ireland, Norway and Denmark, which have also restricted research using human embryonic stem cells. This raises an interesting moral question of whether these nations will allow their patients to receive the treatments developed in the future using technologies that they consider unacceptable.

Belgium, Sweden and Spain allow therapeutic cloning and human embryonic stem cell use in similar frameworks to the UK, and there is now public pressure in Germany and Italy to revisit their legislation, while Ireland is already doing so.

In Asia, the picture is very different. Japan, China, Singapore and South Korea all follow the UK's approach. India is embracing human embryonic stem cell research, as realised recently at an Indo-UK meeting organised by the Royal Society and aimed at spawning international collaborations in the field. But so far it still has a ban on therapeutic and reproductive cloning.

South America is as divided as Europe. Ecuador bans embryonic stem cell research and both types of cloning; Brazil bans cloning, but a new law allows and funds embryonic stem cell research; Argentina, Chile, Peru and Uruguay ban both types of cloning, and legislation either allows or does not cover embryonic stem cells, and only Colombia permits therapeutic cloning as well as human embryonic stem cell research.

A global scientific effort is needed to make the most rapid possible progress. Yet opinions and legislation around the world are deeply divergent.

In the Middle East, only Israel and Turkey have any relevant legislation. Israel permits therapeutic cloning and embryonic stem cell research while banning reproductive cloning. Turkey has effectively the same—although stem cell research is not explicitly permitted, it is just not mentioned.

On the continent of Africa, only South Africa (embryonic stem cell research—yes; both types of cloning—no) and Tunisia (embryonic not specifically prohibited; both types of cloning—banned) have enacted laws.

For the countries that do not have national legislation we can gain an idea of their attitudes from the ill-fated attempts to gain consensus at the European and international levels.

The Council of Europe has introduced the ambiguous European Convention on Human Rights and Biomedicine. It is not clear whether it bans therapeutic cloning. Thirty-one of the 45 member states have signed, of which 15 have also ratified. In response to the

GAUTAM SINGH *AP Photo*

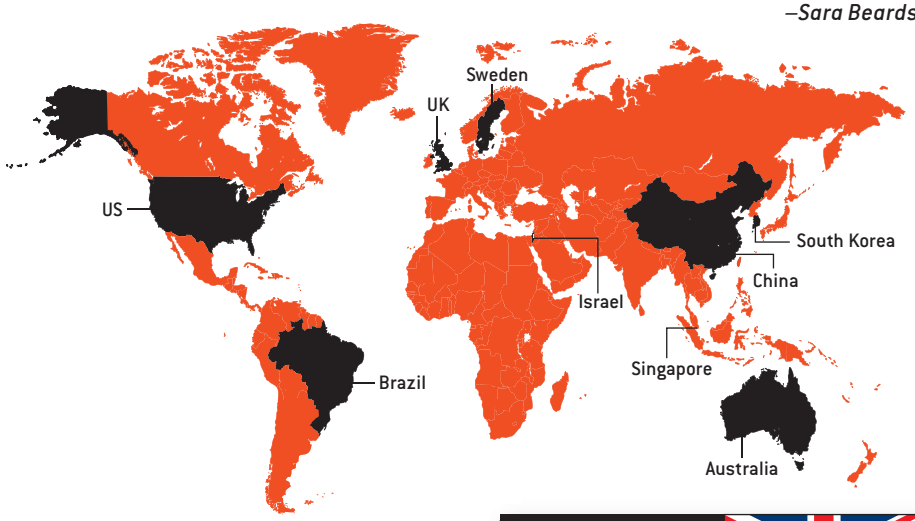
SIR MICHAEL ARTHUR (*right*), British High Commissioner to India, confers with K. VijayRaghavan, director of India's National Centre for Biological Sciences, at a stem cell workshop in April. The UK intends to take some of its stem cell research to India.

A World of Approaches to Stem Cells

Around the globe, stem cell research has met with reactions varying from enthusiasm (as in the UK) to suspicion and distaste. Despite increasingly permissive international laws, no consensus on supporting the research has emerged, even among the selection of "stem cell progressive" countries considered here. The US government, for example, provides an enormous sum ($550m) for stem cell investigations by global standards, but the portion for human embryonic stem cell (hESC) studies ($24m) is only slightly above the spending by countries with much smaller budgets where investments go farther.

Nations also differ on how much regulatory control they choose to exercise. Some have laws that specifically permit or prohibit certain practices associated with hESC work, such as therapeutic cloning, but others keep such experiments in a legal limbo. Critics have raised concerns about the inconsistency of the resulting systems: one scientist notes that EU funding has created a "bizarre situation" in Germany, where scientists can apply for projects that are officially deemed illegal. (Funding figures represent estimates of the current annual spending in US dollars on all types of human stem cell research, except where noted.)

–Sara Beardsley



EU

Production of new hESC lines: Permitted from unused IVF embryos where legal in member nations

Therapeutic cloning: Prohibited

Funding: $170m on stem cells over the past three years (only $650,000 for hESC research)

Status in some member nations:
- **France:** Creation of hESC lines from IVF embryos legal as of October 2004; public funding is $4m
- **Germany:** Only work on hESC lines predating 2002 is legal; public funding is $4m
- **Finland:** Permits research with IVF embryos; public funding is $5m
- **Italy:** June 12 referendum will consider permitting IVF embryo research; public funding is $6m

EU will not increase funding for hESC projects despite a doubling of the total research budget.

US

Number of published hESC lines: 46

Production of new lines: Legal, but prohibited with federal funds

Therapeutic cloning: Legality varies from state to state

Federal government funding: About $550m for all stem cell research ($24m for hESC)

Private funding: About $200m

Public funding at state level:
- **California:** $3bn over 10 years
- **New Jersey:** $11.5m (another $380m proposed)
- **Wisconsin:** $375m proposed
- **Illinois:** $1bn proposed
- **Connecticut:** $20m proposed

Federal government allows its funds to be used only on the 22 available hESC lines created before August 2001.

Pending legislation would relax some of these federal restrictions.

UK

Number of published hESC lines: 3

Production of new lines: Legal

Therapeutic cloning: Legal

Government funding: About $80m

Private funding: $15m–$20m

The Wellcome Trust alone has spent $12m annually since 2002.

First licence for human ES cell research was granted in 1996.

The Human Fertilisation and Embryology Act of 1990 allows the UK to fund hESC research flexibly.

UK's first licence for human cloning research granted in 2004. Its recipients in May announced the country's first cloned human embryo.

SWEDEN

Number of published hESC lines: 8

Production of new lines: Legal

Therapeutic cloning: Legal as of April

Number of researchers: 400

Government funding: $10m–$15m

Private funding: Cellartis and NeuroNova, the two largest stem cell research companies in Sweden, contribute the bulk of the $35m spent annually there

Cellartis, the single largest source of defined hESC lines in the world, maintains more than 30—two of which are approved by the US National Institutes of Health.

JEN CHRISTIANSEN; REPORTING BY SARA BEARDSLEY

BRAZIL

Production of new hESC lines: As of March, legal from IVF embryos at least 3 years old

Therapeutic cloning: Banned

Government funding: $4.5m annually planned, allocated by the Health Ministry and the Science and Technology Ministry

SOUTH KOREA

Number of published hESC lines: 29

Production of new lines: Permitted with case approval from Ministry of Health

Therapeutic cloning: Permitted with case approval from Ministry of Health

Number of researchers: 300-400

Government funding: About $10m

Private funding: About $50m

First to create a hESC line from a cloned embryo. In May the same researchers announced that they had created 11 new hESC lines cloned from patients with spinal cord injuries, juvenile diabetes and a blood disorder.

SINGAPORE

Number of published hESC lines: 1

Production of new lines: Legal, if embryos are destroyed within 14 days

Therapeutic cloning: Legal, as above

Number of researchers: About 150, in industrial and academic settings

Academic spending: About $10m, from public and private sources

Industrial spending: About $10 million

A pending government proposal would spend $60m over the next four years.

ISRAEL

Number of published hESC lines: 1

Production of new lines: Legal

Therapeutic cloning: Legal

Government spending: About $5m

Private spending: $15m–$30m

Israeli scientists led one of the research teams that first isolated hES cells. They were also the first to show that hES cells could be changed into heart cells, and to show that hES cells can integrate with tissues.

CHINA

Production of new hESC lines: Legal

Therapeutic cloning: Legal

Number of researchers: 300–400

Public and private funding: About $40m

The journal *Nature* reports that "China has probably the most liberal environment for embryo research in the world", with little public opposition to such studies. No laws govern stem cell research, but the recommendations of the Ministry of Health endorse it.

AUSTRALIA

Number of published hESC lines: 1

Production of new lines: Conditionally legal

Therapeutic cloning: Banned

Number of researchers: 200–250

Government funding: The Australian Stem Cell Centre has $90m to spend through 2011.

debate in the UK, which preceded the introduction of its legislation on cloning, an additional Protocol on the Prohibition of Cloning Human Beings was drafted to try to influence the outcome. Unsurprisingly, the UK has not signed either, but as neither the convention nor the protocol gives any sanctions for violation it is unlikely to have any major effect. Portugal, though, has signed and ratified the convention, despite no national legislation, which is a likely indication of its views.

At the United Nations we see a similarly confused picture. A committee was formed in 2001 to consider "the elaboration of an international convention against the reproductive cloning of human beings". Four years of stop-start debate and negotiations saw member states unable to get anywhere near a consensus on whether therapeutic cloning should be included in the ban.

One of the most influential groups during the tail end of the debate was the Organisation of Islamic Countries (OIC). It is suspected that part of the reason that those seeking a ban on all forms of cloning, such as the US and Costa Rica, did not push for a convention was because of a last-minute indication that the OIC would support an alternative proposal. Initiated by Belgium and supported by the UK, the proposal asked that individual countries be allowed to make their own decision on therapeutic cloning.

Instead the result was a poorly worded and ambiguous political declaration that appears to ban all forms of cloning. But because it is nonbinding, it will have absolutely no effect on countries that wish to forge ahead with therapeutic cloning.

Unfortunately, this outcome also means that no clear message has been sent to maverick scientists that the entire world believes that reproductive cloning is unacceptable.

Richard Gardner is chair of the Royal Society's working group on stem cell research and cloning. Tim Watson (tim.watson@royalsoc.ac.uk) is a press officer at the Royal Society.

Stem Cells: East ...

Country Report: CHINA

Generous staffing and permissive laws aid Asia's largest stem cell effort

China has Asia's most extensive stem cell research effort, with a particular emphasis on driving innovative adult stem cell therapies toward clinical trials. Although it is hard to find statistics that pull together China's fast-growing patchwork of stem cell initiatives, the country must have at least 300 researchers in the field, working in 30 separate institutions.

A delegation sent late last year by the UK Department of Trade and Industry to look at stem cell research in Asia visited a dozen Chinese labs and concluded: "The facilities were, in every case we saw, equipped, funded and staffed to levels at least as good—in most cases better—than equivalent centres in the UK". Chinese stem cell labs have plenty of well-motivated junior staff, many of whom have returned from postgraduate training in Europe and North America. The senior researchers, who have also worked abroad, are providing strong leadership, but there seems to be a temporary gap in the middle, among the cadre of postdoctoral scientists who form the background of the scientific effort in the West. China has a few rudimentary stem cell companies, but commercialisation is still at an early stage.

Like their counterparts elsewhere in Asia, Chinese stem cell researchers benefit from an ethical and regulatory environment that is generally more favourable to stem cell research than in even the most permissive Western countries. "The status accorded to the embryo is similar to that in the UK, but regulations are operated in China with a fairly light touch", says Genevra Richardson, professor of public law at Queen Mary, University of London. "Most ES research teams in China use fresh embryos".

LINDA WELLS (*center*) of Albuquerque, NM, watches as a technician inspects a stem cell sample at a laboratory in Tianjin, China. Wells went to China after doctors discovered stem cell samples from a Chinese child that would provide a match for her daughter, Kailee, who suffers from aplastic anemia.

GREG BAKER *AP Photo*

China is well represented in embryonic stem cell work, with at least 10 ES cell lines established in the country—and is working on therapeutic cloning. "China has better access to human oocytes than we have in the West—and fantastic nuclear transfer skills", says Peter Mountford, chief executive of Stem Cell Sciences, based in Edinburgh. "There are many extremely dextrous hands available to manipulate those tiny dots [human eggs]".

But the Chinese scene is still dominated by adult stem cell work. "There is a very significant focus on clinical translation, which is much more palatable in China than in the US or Europe", says Stephen Minger of King's College London. "Treatments will be pushed ahead more quickly than in the West".

A colourful example is Jianhong Zhu of Huashan Hospital, part of Shanghai's Fudan University. He is working with adult neural stem cells, extracted from brain tissues exposed in patients who suffer open head wounds. (A classic local example is the "chopstick injury", in which a barbed bamboo chopstick is pushed—usually through an eye socket—into the head during an argument over a meal; when the stick is removed, enough brain tissue sticks to it to be a source of neural stem cells.) Zhu has obtained encouraging results from a clinical trial in which eight such patients had their own neural stem cells cultured and transplanted back into the site of their injury; they fared significantly better than eight matched controls who had open brain surgery but no cell grafting.

—*Clive Cookson*

... and West

Country Report: UNITED KINGDOM

Positive public attitudes lift British scientists above the destructive fray

When the international stem cell research race got started at the end of the 1990s, two factors put Britain in a strong position. One was the historical strength of embryology and related sciences in the UK, the other its well-established regulatory framework.

Any researcher working with early human embryos owes an immense scientific debt to Patrick Steptoe and Robert Edwards, the British pair who developed the IVF techniques that led to the birth in 1978 of Louise Brown, the world's first test-tube baby. That led to an intense debate about the ethics of using "spare" embryos for research, culminating in 1984 with Mary Warnock's landmark official report that recommended allowing controlled research on human embryos up to 14 days after fertilisation—a limit that remains a de facto world standard.

Warnock's conclusions were enshrined in law six years later, with the establishment of the Human Fertilisation and Embryology Authority to regulate the field. So, when human ES cells and cloning came along, it was relatively straightforward for the UK to amend its legislation to allow research for therapeutic purposes on cells derived from human embryos (including cloned embryos) while banning reproductive cloning. Two therapeutic cloning projects are already under way, at Newcastle University and the Roslin Institute.

Although Britain has a vocal anti-abortion lobby opposed to embryo research, it is very much in the minority. In the UK, unlike many other countries, stem cells and cloning are not party political issues. Stem cell researchers who have come to Britain from other countries, such as Roger Pedersen to Cambridge and Stephen Minger to King's College London from the US and Miodrag Stojkovic to Newcastle from Germany, emphasise the importance of the supportive public and political attitude to their work.

KING'S COLLEGE LONDON

INVESTIGATOR at Stem Cell Biology Laboratory at King's College London works with human embryonic stem cells.

The positive attitude of the UK government—and even more enthusiasm from Scotland, which has set out with some success to become a regional hotbed of stem cell science—has already given Britain a good research infrastructure in this field. It has the world's first stem cell bank, which is leading an international initiative to characterise all the ES cell lines now available around the world, identify their salient features and assess the degree of diversity that different lines may exhibit.

Still, the public funding position for stem cell research in the UK is not so rosy by international standards. In 2002 the government announced a £40m ($70m) investment in stem cell science by the country's research councils—and, although this has been supplemented with some further funds, Britain's financial commitment falls short of some of its competitors in the Asia Pacific region as well as individual American states.

Although Britain is home to a few small stem cell companies, such as ReNeuron and Stem Cell Sciences, there is little investment from traditional private sector sources such as venture capitalists and fund managers who see the field as too long-term and risky [see "Tough Cell to Investors," on page A32]. In an attempt to fill the funding gap, a powerful group of scientists and business people has set up the UK Stem Cell Foundation, a nonprofit organisation that aims to raise £100m to support the development of stem cell therapies, in collaboration with existing government and charitable programmes.

—Clive Cookson

The California Gambit

Biologists applauded the Golden State's $3bn wager on stem cell science. But as W. Wayt Gibbs reports, the stakes may be higher than they realise

Last November, Californians elected an action hero to fix their broken budget and simultaneously agreed to borrow billions for a massive taxpayer bet on long-shot research into embryonic stem cell therapies. This is clearly not a state for the risk averse. But by rushing in where Congress feared to tread, Californians initiated a policy experiment—or a political end run—with national repercussions. Even as many stem cell biologists revel at their good fortune, some worry that this seismic shift in policy could fragment the field, delay scientific progress and raise unrealistic expectations among the public. The scale of these risks is not yet clear.

What is clear, at least to most scientists in the field, is that the previous system was not working. Under rules laid out by President Bush, researchers cannot use funding from the National Institutes of Health or other federal agencies to experiment on any of the 200-odd lines of human embryonic stem (ES) cells derived since August 2001, when the rules went into effect. Unfortunately, all of the 22 ES cell lines created before that date have been contaminated by nonhuman molecules that invite immunological attack, which greatly limits their medical use.

"There is no question that the NIH attitude and political climate had cast a real chill on this area", says Arnold Kriegstein of the University of California at San Francisco. To work around the federal restrictions, UCSF created a stem cell research programme in 2002 with $5m (£2.7m) donated by former Intel chairman Andy Grove and hired Kriegstein to run it. Stanford University set up a similar programme with a $12m anonymous donation, and last year Harvard University joined the fray with its own private stem cell institute.

Despite these efforts, Kriegstein says: "It is difficult to get involved in a field where research you may want to do may be criminalised at some time in the future". (Indeed, in some states, such as Arizona and Pennsylvania, deriving a new stem cell line from human embryos is already a felony.)

"For a young investigator starting a new lab, focusing on embryonic stem cells involves enormous risk", says Melissa Carpenter, who directs stem cell biology at CyThera in San Diego. "If the NIH decides to cut you off, then where will you be? It's an extreme shame. I know a number of good scientists who avoid the area altogether because it is so ethically charged".

As a result of the federal freeze, says Mahendra Rao of the National Institute on Aging, "the US has ceded leadership in this new field to other countries. When we talk about new markers and antibodies to identify stem cells, we point to work done in England. For progress in bioprocessing and scale-up, we look to Israel or Singapore. I now go out of my way to attend scientific meet-

STEFANI OKASAKI AND TIM HARRISON (*UCSF*); LEE SNIDER/PHOTO IMAGES/CORBIS (*Harvard University*); DAVID MCNEW/GETTY IMAGES (*Arnold Schwarzenegger*)

2002 // 2004

AUG 2002
University of California, San Francisco, launches a $5m stem cell biology program

DEC 2002
Stanford University creates a stem cell research centre with a $12m anonymous donation

MAR 2004
Douglas Melton of Harvard University creates 17 new ES cell lines with private funds

APR 2004
Harvard launches its stem cell institute

JUN 2004
Stem Cell Research Enhancement Act is introduced into the US House but never makes it to a vote

NOV 2004
Proposition 71 passes in Calif., clearing the creation of a 10-year, $3bn Institute for Regenerative Medicine (CIRM)

Wis. governor Jim Doyle proposes devoting $375m over 10 years to a new research institute for stem cell biology and other medical research. Doyle also proposes giving $75m over five years to state medical schools for research, including on stem cells

UNGER KEVIN/CORBIS SYGMA [*petri dishes*]; ANDREA PISTOLESI/IMAGE BANK/GETTY IMAGES [*tractor*]

ings in China in order to hear new and unpublished work". Many biologists are frustrated, Rao says, "because the US still could easily be the leader in this kind of science. These cells were discovered here, and we have the best infrastructure for analysing them. We just haven't figured out how to put together the policy to do it".

That is precisely the problem that California aims to solve. California's answer to the president's restrictions is its new Institute for Regenerative Medicine, CIRM. Created by the 59 per cent of voters who favoured Proposition 71 on last November's state ballot, the institute is to be governed by a small staff of about 40 scientists (only three of whom had been hired by the end of April), a handful of administrators, and an oversight committee of 29 academics, businesspeople and medical activists. Its purpose is to spend $300m a year on stem cell research for a decade, an unprecedented growth spurt for a field so nascent and so controversial.

The move set alarms ringing in dean's offices and state legislatures around the country. The governors of Wisconsin and New Jersey quickly launched campaigns to boost stem cell research funding for their state universities. Lawmakers introduced bills legalising human ES cell experiments in biotech-heavy states such as Maryland and Massachusetts [*see timeline below*].

"When Prop 71 was passed, we became anxious that it would be difficult to attract talented leaders to Connecticut for our own stem cell research programme", says Robert Alpern, dean of the Yale University School of Medicine. He and others have persuaded the governor to support a bill that would condone work with certain human ES cells and would provide $10m a year for stem cell science. So far, Alpern reports, the bill faces no organised opposition but has yet to reach a vote.

"Human ES cells are so new, and few people are trained to use them properly to do good, innovative experiments on how they grow and differentiate. In the US there are just a few dozen people at most", observes Gordon Keller, a stem cell biologist at Mount Sinai School of Medicine in New York City.

The competition for these people is rising fast, Kriegstein says. In addition to international demand, "lots of institutions in California are trying to build or strengthen programmes right now, and they are all looking at the same candidates. That may increase the cost of attracting the best people", he states.

Keller worries that "if you funnel too much money into a field that doesn't yet have enough talent to absorb it, it is

FRAZER HARRISON/GETTY IMAGES

ELECTORAL CAMPAIGN for Proposition 71 succeeded, but the research campaign for stem cell therapies is just beginning.

going to be wasted". CIRM's interim president, Zach Hall, plans to address that problem by using the institute's initial rounds of grants to train more scientists and build more labs. (NIH restrictions prohibit work on unapproved human cell lines in any lab that runs on federal funds.)

In the first round, "the intent is to encourage institutions to put together coherent training programmes for stem cell science", Hall says. Organisations will compete for 18 awards to be announced in late 2005 that will provide up to $1.25m a year, depending on the size of the training initiative. Although some of the $15m a year will go toward student stipends, Hall notes, the grants cannot pay for

2005

JAN 2005
Scientists report that all NIH-approved human ES cell lines have been contaminated by foreign antigens

NJ governor Richard Codey proposes raising $380m for a state stem cell institute

NY state senator David Paterson proposes the creation of a stem cell research programme funded with $1bn over 10 years

FEB 2005
Mass. governor Mitt Romney urges state legislators to criminalise the creation of new human stem cell lines for research

Stem Cell Research Enhancement Act reintroduced to Congress, with 186 sponsors in the House and strong support in the Senate

MAR 2005
UCLA launches an institute for stem cell biology

Calif. Supreme Court dismisses two lawsuits challenging the constitutionality of CIRM

Ill. comptroller proposes to issue $1bn in bonds and to tax cosmetic surgeries to fund a state stem cell institute

APR 2005
Md. state senate kills a bill, which had passed the House, to create a stem cell research programme with $23m a year in state funding

MAY 2005
Mass. legislature passes a bill permitting ES cell research with enough votes to override an expected veto by the governor

AUG 2005
Construction to begin on $150m stem cell research centre in New Jersey

AUTUMN 2005
CIRM plans to award its first grants

Scientists Follow the Money

A brain drain out of the US turns into a gusher for California

Shortly after President Bush announced in August 2001 that federally funded stem cell biologists in the US would have to work under tight restrictions, Roger Pedersen packed his bags for the UK. Pedersen, whose research at the University of California at San Francisco had earned him a place near the top of his field, moved his lab to the more liberal environment of University of Cambridge.

Leaving the US proved to be a good career move for Pedersen: last year Cambridge made him co-director of a new $30m stem cell institute. And Pederson was hardly alone in his emigration, observes Mahendra Rao, who directs stem cell research at the US National Institute on Aging. Rao points to several scientists who left lucrative biotech posts in the US to set up lab-keeping overseas.

But if there was a brain drain of stem cell investigators from the US, the attraction of a $3bn honeypot in California seems to be reversing the flow. "A number of leading scientists in our field have been interviewing in California for lead positions", says Melissa Carpenter, an American pioneer in the field who jumped two years ago to the Robarts Research Institute in Ontario, Canada. "UC Irvine is recruiting aggressively", Carpenter reports, "and so is Stanford". Carpenter herself just decided to return to the US to head up stem cell research at CyThera, a startup in San Diego. The passage of Proposition 71 was not the only reason for her return, she says, but it was an important factor.

Indeed, the Golden State is beckoning to many in the field, including those elsewhere in the US. At the National Institutes of Health, Rao says "it has been getting harder to recruit, and we are losing people [to California]". Arlene Chiu, who directed a stem cell research programme at the NIH, quit in April to take a job with the new California Institute of Regenerative Medicine (CIRM). James Battey, the current director of the National Institute on Deafness and Other Communication Disorders, says he has applied to CIRM for the job of president.

"We cannot compete by giving them more money", Rao explains. "And many people have a real worry about federal funding being available in the future. I myself have been tempted" to join the California bandwagon, he admits.

Although the westward pull is strongest for senior researchers, it seems to be influencing young scientists as well. "We have recruited a group of students for next year", says Arnold Kriegstein, who leads a stem cell training program at UCSF. "I think Prop 71 made some of them choose UCSF over institutions back east".

"The US is competing with Singapore, Australia, the UK—there are considerable resources there, too, and the restrictions are considerably fewer", Carpenter says. "Before joining CyThera, I looked at those as options for myself", she adds. "It's definitely a competition, and it will be interesting to see how it all falls out". —*W.W.G.*

MATT COLLINS

PhD programmes, and no school will receive more than one grant.

So when will the California money start flowing to do actual science? Hall cannot answer that question yet, as the agency must first clear several significant obstacles. Six months after its birth, CIRM was still without permanent offices, a permanent president, a slate of experts to review research proposals, or authorisation to issue the bonds from which it will draw its budget.

The bonds were hung up by a pair of lawsuits that challenged the legitimacy of CIRM. In March the California Supreme Court declined to hear the suits but left plaintiffs with the option of bringing them to lower courts. One of the suits, by two pressure groups called People's Advocate and the Life Legal Defense Foundation, landed before a superior court in April. It asserts that the new institute violates a provision in the state constitution. A CIRM official says that the state finance committee might approve bonds to raise money for the institute before the legal dispute is settled.

Even before the money valve opens, scientists could start sending in their requests for research grants. But the institute must seat a panel of 15 stem cell experts from outside California to conduct peer review of the proposals. This is no small feat. Many researchers in the field are being recruited to California [*see box above*] and thus have a conflict of interest. Among those who are qualified, few may be willing.

"I've been asked by CIRM to sit on various panels", Keller says. So far he has declined. "We already do a lot of

reviewing for NIH, from which we also draw funds. When they ask us to do the same for California but don't allow us to apply for their money … well, there are only so many hours in the day".

Ironically, in setting themselves up for financial success, the state's researchers have also set themselves up for possible political failure. By emphasising medical breakthroughs (as Richard Nixon did in the "war on cancer") rather than technical milestones (as Francis Collins did in the Human Genome Project), the campaign for Proposition 71 placed a sizeable bet on an uncertain outcome.

"Science is being put under its own microscope", reflects Fred Gage, a neuroscientist at the Salk Institute. "We are going to be accountable for coming up with major discoveries. There clearly is an expectation that before the end of the decade there will be financial as well as therapeutic benefits to the state".

At stake, too, are precedents of national importance. California's action appears to have spurred support for the Stem Cell Research Enhancement Act, a bill that died in the US Congress last year but was resurrected in February. Republican leaders have promised to put the bill to a vote this summer. Were it to pass and survive an expected presidential veto, it would remove the August 2001 restrictions on federally funded stem cell research, freeing the NIH to compete with private and state initiatives on a level pitch. The law could also be a boon to CIRM, however, because it would allow the agency to spend less on scientific construction and equipment and more on the science itself.

Ultimately, if the California gambit succeeds—whether politically, economically or scientifically—it could become a new model for funding those kinds of research that offend the majority in some parts of America but enthrall most people in other regions. That may not be the most efficient way to do science, but it might yet prove to be the most expedient.

STANFORD UNIVERSITY/GETTY IMAGES

The Ghost of Lysenko

Biologist **Irving Weissman** warns of the cost of irrational restrictions

By many measures, the US leads the world in biomedical discoveries, technologies and therapies. Recombinant DNA technologies for genetic manipulation were born in America and have produced a multitude of drugs and diagnostic devices by means of a new commercial entity, the biotech startup.

At a critical stage in US history, federal and local governments nearly banned recombinant DNA technology. But instead new regulations required academic and commercial research entities to submit their plans for approval to national and local advisory committees—and research prospered. This kind of regulation, which preserves the essence of unfettered research with the least intrusive bureaucracy and meaningfully protects scientists and society, could be called the American way. Pioneering research moves forward while society continually monitors and receives the benefits by translating discoveries into patient care.

History shows the folly of more oppressive interventions. Trofim Lysenko was a maverick biologist who convinced Josef Stalin in the 1920s that the Darwinian view of natural selection was wrong. Darwinian genetics consequently had no home in Russia for decades, while American agriculture and medicine prospered, very significantly aided by migrant Russian geneticists. The Russian way, then, held that ideology trumps science, leading to the loss of good science for generations.

The spectre of Lysenkoism haunts the US debate over stem cells. Because the isolation of stem cells from an embryo ends the possibility that it could be implanted in a uterus, people who feel any biological entity beyond fertilisation is human think this research is immoral. That view underlies the bills by Senator Sam Brownback of Kansas and Representative Dave Weldon of Florida that criminalise this practice.

As part of the administration's current policy that restricts federally funded use of stem cell lines to those made before August 2001, President Bush included a funding ban on production of pluripotent stem cells derived by nuclear transfer, which some call therapeutic cloning. The Weldon/Brownback bills would criminalise that practice, effectively limiting such research to non-US science. Thus, ideology has severely curtailed a foundation technology critical for rapid advances in human developmental biology, an understanding of the causes of human disease and development of potential human therapies. (The Weldon/Brownback bills are not law because a bipartisan coalition in the Senate has blocked their passage.)

Who loses from this federal ban? Not just life science research; not just the young scientists who wish to spend their lives pushing scientific frontiers for knowledge and for therapies. Most of all, it is the tens of thousands of patients who might have been helped. Which is the higher moral ground: saving the world from "therapeutic cloning" or saving the lives of the sick?

Fortunately, consistent with its constitutional right, in 2002 California passed bills to encourage and regulate embryonic stem cell and therapeutic cloning research. In November 2004 the state passed, by a 59 to 41 margin, a $3bn initiative to fund this research over 10 or more years. California has taken on the task of funding mainly basic research in these areas. The timelines to therapies are essentially what should be expected if the National Institutes of Health had funded this research.

While many people think it is a serious problem to substitute state for federal funding of science, I am not among them. I hope that this current intrusion of religion and ideology into federal research is only a transient aberration, but the lessons from the Lysenko experience tell us this situation could last a long time.

—Irving Weissman is professor of pathology and developmental biology at Stanford University, director of the university's Institute for Cancer/Stem Cell Biology and Medicine, and a co-founder of StemCells, Inc., and Cellerant, Inc, both in Palo Alto, Calif.

Growing Pains for the

ES Cell International

In Singapore, a company with ambitious goals leads a "privileged existence"

Singapore-based ES Cell International (ESI) has emerged as one of world's first commercial ventures to focus on developing stem cells for therapeutic purposes.

Established in 2000, ESI sought to draw on the pioneering research of Ariff Bongso and other researchers at the National University of Singapore in growing stem cell lines from human embryos. As part of Singapore's quest to become a global centre of medical research, the government's Economic Development Board agreed to finance ESI in co-operation with several wealthy Australian investors.

The company received a boost in 2001 when ESI was among 10 groups selected by the US National Institutes of Health to have stem cells eligible for federal funding under the Bush administration's stem cell plan. But ESI's original business plan to produce and sell human embryonic stem cell lines promised to produce only "minimal" profits of around $300,000 (£160,000) a year, according to Alan Colman, ESI's new chief executive.

Colman, who gained fame as head of the research team that cloned Dolly the sheep in Scotland, joined ESI in 2002 as its chief scientist with the aim of turning stem cells into treatments for a range of illnesses. One project is to try to induce stem cells to turn into insulin-producing "islet" cells that could be implanted into diabetics.

ESI works closely with researchers from Australia's Monash University, Israel's Hadassah University, the National University of Singapore and the Netherlands' Utrecht University, with the first three holding an 18 per cent stake in the company. ESI would serve as the exclusive worldwide licensee of any resulting patents from their research. ESI has an ambitious goal to gain approval from the US Food and Drug Administration by 2010 for products derived from stem cells that would combat diabetes and heart diseases.

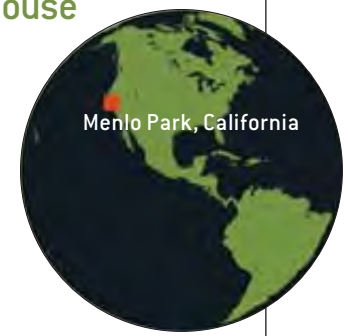
"We have a privileged existence", declares Colman, referring to the financial support given by the Singapore government, which holds a 44 per cent stake in ESI.

Nonetheless, he is worried about whether that support will last long enough for ESI to reap commercial benefits from its research work. "Singapore appears to be shifting its biomedical financing from applied research with start-ups to basic research", he says.

Although ESI has raised a total of $24m in the form of equity investments and loans since 2000, its annual cash "burn" amounts to $3.6m. For ESI, it is a race against time. —*John Burton*

Geron

The former patent powerhouse works on new therapies



California-based Geron was once feared for its patent might. Because the company held exclusive rights to many embryonic stem cells developed at the University of Wisconsin, biotechnology rivals believed the company would establish a stem cell monopoly. In 1999 Geron purchased rights to the cloning technology used to make Dolly the sheep in Scotland, a technique given patent protection by the British government a year later.

The controversy over Geron's extensive patent holdings only subsided in 2002 when the company and the University of Wisconsin reached an agreement that limited Geron's patent rights and promised to allow other scientists access to the stem cell lines.

Today the company is still operating at a loss—$9.7m (£5.2m) in the first three months of 2005—and fears of its domination of the stem cell market have evaporated. Yet Geron is still an important force in this area of research and is expected to be one of the main beneficiaries of a new California fund for stem cell research.

Geron, founded in 1992, was one of the first public companies to study embryonic stem cells. In the late 1990s its attention turned to telomerase, a compound the group identified through its study of stem cells as key to the aging process. Cell levels of telomerase decline as humans age. Geron scientists hope that by boosting amounts of the compound in the body, they can battle diseases such as AIDS and cancer. In March the company founded TA Therapeutics, a joint venture with a Hong Kong University research institute, to explore telomerase applications.

But Geron's interest in embryonic stem cells as a therapy in their own right has been renewed. The company is pursuing research in a wide number of disease areas, including Parkinson's, heart disease, diabetes, arthritis, blood disease, osteoporosis and organ transplantation. While none of the therapies has been tested in humans yet, Geron says it may soon initiate clinical trials in spinal cord injury.

In March the company published research explaining how human embryonic stem cells could be grown without the help of "feeder cells". Feeders such as mouse cells were used to propagate early stem cell populations. Geron had posted research on how to grow the cleaner embryonic stem cells on its Web site in September of 2002, but until this year's publication in the journal Stem Cells, many had doubted the technology really worked.

—*Victoria Griffith*

LUCY READING-IKKANDA

New Industry

STEM CELL CORPORATE LEADERS

ES International www.escellinternational.com	ALAN COLMAN, ESI's chief executive, wants to try to induce stem cells to turn into insulin-producing "islet" cells. Colman had an accomplished career as an academic, which included research and teaching appointments at the University of Oxford and the University of Warwick and the appointment of professor of biochemistry at the University of Birmingham.	
Geron www.geron.com	THOMAS OKARMA, Geron's chief executive, plans to lead his company soon into clinical trials of stem cells for spinal cord injury. Okarma holds an AB from Dartmouth College and an MD and PhD from Stanford University.	
ACT Holdings www.advancedcell.com	MICHAEL WEST, ACT Holding's chairman and president, shifted the corporate focus to embryonic stem cell research. West received an MS in biology from Andrews University in 1982 and a PhD from Baylor College of Medicine in 1989. West recently relinquished the chief executive's position to William M. Caldwell IV (*not shown*).	
Stem Cell Sciences www.stemcellsciencesltd.com	PETER MOUNTFORD shepherds a business plan to commercialise ES cells, first as a research tool and later as cell-based therapies. He received a doctorate from Melbourne University and was a Royal Society (London) Endeavor Fellow at the University of Edinburgh. He is the inventor of technologies that have been widely adopted in stem cell research.	

POLAK MATTHEW/CORBIS SYGMA (*Colman*); GERON CORPORATION (*Okarma*); ACT (*West*); STEM CELL SCIENCES (*Mountford*)

Stem Cell Sciences

Once a "virtual company", it has grown over a decade into the most international force in stem cells

Stem Cell Sciences must be the most global of any stem cell company. SCS has corporate research and development centres in the UK, Japan and Australia and plans to set up a US operation this year. Its bold business plan is based on commercialising human embryonic stem cells, first to sell as a research tool to the pharmaceutical industry and later to develop cell-based therapies.

Peter Mountford, the chief executive, set up SCS in his native Australia as a "virtual company" in 1994, shortly after returning home from a productive period working in Scotland with Austin Smith, the Edinburgh stem cell pioneer. In 2000 it became a real company with employees and staff in Melbourne, and the following year Mountford set up a Japanese operation, SCS KK, in Kobe, where it collaborates with stem cell researchers at the RIKEN Centre for Developmental Biology.

In 2003 Mountford moved back to Scotland and set up SCS's corporate headquarters in Edinburgh. Mike Dexter, a stem cell biologist who had just completed a five-year term as director of the Wellcome Trust, became company chairman. Mountford was attracted by Scotland's emergence as a centre of excellence in stem cell research and above all by the prospect of working again with Smith, who now runs the Institute for Stem Cell Research at Edinburgh University.

SCS directly employs about 40 people—half in Japan and the others divided between Scotland and Australia. Over its lifetime, the company has raised about £5m ($9.25m) from investors and another £5m through collaborative research and licensing deals with pharmaceutical companies, including Pfizer, GlaxoSmithKline and Aventis. Stem cell therapies lie further in the future, with Parkinson's disease one possible target.

While Mountford has nothing but praise for Scotland's scientific credentials and the encouragement his company has received from government bodies such as Scottish Enterprise and the UK Department of Trade and Industry, he is critical of Britain's venture-capital community for failing to see the long-term value in SCS.

The next funding round will focus on American investors, with a possible listing on London's Alternative Investments Market, to raise money to start a US operation. The location for the US development centre has yet to be decided. Mountford says the long-term aim is a Nasdaq listing in New York City, although he wants to keep the corporate headquarters in Scotland. *—Clive Cookson*

ACT Holdings

The tiny company that ignited a political battle over human therapeutic cloning continues to punch above its weight

ACT Holdings has long received attention out of proportion to its size. The tiny biotechnology company employs just a couple dozen people in cramped offices in Worcester, Mass.

The group has attained notoriety for its work in human therapeutic cloning. In 2001 Advanced Cell Technology (ACT), as it was then known, announced it had cloned a short-lived human embryo, igniting a political battle in the US Congress over the practice. In March the British science journal *Lancet* reported that the company had created human embryonic stem cells without using cell "feeders", about the same time rival Geron published similar research. The breakthrough is important because exposing stem cells to mouse or human cell feeders contaminates them, rendering them potentially unusable for medical therapies.

Despite the controversy and excitement surrounding its science, ACT has always operated on a shoestring. Its executives have publicly lamented their tight budgets, saying they have often had trouble paying their small staff.

With a new name and management structure and fresh plans to expand to California, the group is hoping for renewed corporate life. In February the company went public in a "reverse merger" into the shell of a publicly traded group, Two Moons Kachinas. The Utah firm was founded in 2000 to sell Native American ceremonial dolls originally used to promote fertility. The collectible dolls have been forgotten, but the deal allowed ACT to avoid the high cost of an initial public offering.

The group has a new CEO: William Caldwell IV. Former CEO Michael West—who in 1998 left Geron, which he founded, to head ACT—has become chairman and president. At the time of the merger, the company received a much needed infusion of cash, $8m from venture capitalists and private investors. The company hopes its new standing will help it raise even more money. ACT Holdings trades over-the-counter.

While ACT says it will stay in Massachusetts, the company plans to set up a satellite research facility in California to take advantage of the just approved $3bn programme to finance stem cell research.

ACT was founded in 1994 for the purpose of cloning livestock and transgenic animals used to make human medicines in their milk. Although the company still works with animal cloning, the focus shifted under West's leadership to human embryonic stem cell research. The company says it will not pursue cloning for the purpose of reproduction and is only interested in using the technique for regenerative medicine. *—Victoria Griffith*

LUCY READING-IKKANDA

Tough Cell to Investors

Venture capitalists fully understand the rich potential of stem cells. Yet a host of reasons also makes them hesitate to invest, as Nuala Moran explains

Not only is stem cell research the most politicised field in the history of science, it is also one of the most dauntingly complex. So while stem cells have the potential to provide therapies for a vast range of ills, it is proving hard to attract the investment needed to develop them.

Many venture capitalists make the comparison with monoclonal antibodies, which took more than 20 years to translate from basic research to marketed products. As Lutz Giebel, venture partner at SV Life Sciences in San Francisco, remarks: "The promise of monoclonal antibodies was obvious, but VCs [venture capitalists] that invested at an early stage pretty much lost their shirts".

Not that stem cell companies are entirely unattractive. In the first biotech initial public offering of 2005, ViaCell, Inc., a specialist in umbilical cord stem cells, raised $52.5m (£28.4m).

At the point that ViaCell went public, it had annual revenues of $36.8m from umbilical cord blood banking, combined with a cord stem cell product in the clinic, and the potential for forming corporate partnerships. But there are few similar opportunities where the risks inherent in the science are mitigated by a healthy revenue stream.

"ViaCell exemplifies how a lot of VCs feel about the risks of investing in stem cells", says Denise Pollard-Knight, head of Nomura Phase4 Ventures, the VC investment arm of the investment bank Nomura International plc, which was one of ViaCell's major venture-capital backers. "You just have to look at the numbers. VCs have invested $300m to date into stem cell companies as a whole, versus $20bn into other technology platforms".

In many respects this is due to the preliminary nature of the science. G. Steven Burrill, CEO of Burrill & Company in San Francisco, a life sciences merchant bank, says that a VC funding a stem cell company now would be paying for basic research that would ordinarily be carried out in academic laboratories. "We are beginning to see some business plans for stem cell companies, but we are still in the science end of it", he states.

This lack of basic research creates a major risk because it is not clear where the intellectual property might go, says Paul McCubbin, head of Ventures at BTG plc in London. "In the current model if you screen against a receptor and get a hit, you have novel IP; when you stimulate differentiation of stem cells, you have no idea whose IP you might cross", he explains.

Brian Kerr, director at Scottish Equity Partners (SEP) in Glasgow and sees almost every life sciences opportunity in Scotland, examines hundreds of business plans each year. Despite Scotland's scientific standing in the field, SEP has yet to fund a stem cell company. Kerr objects that not only is the science too preliminary, but the business plans per se are too risky.

"Businesses need to be more sophisticated about how they control risk", he says. Stem cells have not been developed as a platform, and too many com-

CORBIS

panies are focusing on a single treatment for a specific disease. "You wouldn't back a conventional science company that had only one product", observes Kerr.

"We are beginning to see some business plans for stem cell companies, but we are still in the science end of it".
—G. STEVEN BURRILL

On top of this he believes a further obstacle has developed in Europe, where the funding engine has broken. After the genomics boom and bust, the public markets have continued to shun biotechs, forcing VCs to fund companies for longer. "It's almost impossible to make money in Europe with a first-round investment in any sort of biotech", states Kerr.

The situation in Europe contrasts with Australia where a number of stem cell companies have listed on the Australian Stock Exchange. But Alison Coutts, director of the investment bank eG Capital in Sydney, says these tend to be early stage: "I think Australia is unique in this respect. While there has been a lot of criticism of the Australian Stock Exchange that it lets companies list 'too early'—quite often when there have been no clinical trials on any product—it has been the primary mechanism for funding a lot of great science that we produce here, and it has even started to attract international companies".

Stem cell startups may also get a sympathetic hearing from Bio*One Capital, the investment arm of Singapore's Economic Development Board. "The potential of stem cell research is too enormous for us to ignore", says Swee Yeok Chu, CEO. "We recognised that we need to take a long-term approach in this field". Bio*One Capital mitigates risk by investing in companies at different stages of development, with different research projects and business models.

That public expectations of the ability of stem cells to provide cures for degenerative disease and severe trauma have gotten so far ahead of what the science can to deliver is largely because of the publicity given to small-scale trials with adult stem cells.

But while there is evidence of efficacy, adult stem cells are not attractive to VCs, says Giebel of SV Life Sciences:

BURRILL & COMPANY

"You just have to look at the numbers. VCs have invested $300m to date into stem cell companies as a whole, versus $20bn into other technology platforms".
—DENISE POLLARD-KNIGHT

"Most people are talking about autologous transplants using cells harvested from patients. But from an investment point of view that's not scalable. It's also difficult for the FDA to get an arm around it. Every single time it is different cells".

While much legwork remains, embryonic stem cells conversely do have the potential to be produced to Good Manufacturing Practice standards.

One VC who has intimate experience of the difficulties of producing potentially commercial stem cell lines is Sir Christopher Evans, founder and chairman of Merlin Biosciences in London. Merlin put £250,000 ($460,000) seed capital into ReNeuron Ltd when it was formed in 1997, followed by £5m a year later. The company went public in November 2000, raising £19.5m and becoming the only quoted stem cell company in Europe.

But ReNeuron was beset by genetic instability problems in its foetal neural stem lines, and in 2003 the Merlin Consortium put fellow investors out of their misery, paying £3.6m to make ReNeuron private again.

The company has since overcome problems with the cell lines and is aiming to get regulatory approval before the end of 2005 (in either the US or the UK) to carry out a clinical trial.

"We have had to pay for work that would normally be done in an academic laboratory, but if ReNeuron came to us today we'd back it again. But as for backing any other stem cell companies—there aren't any", remarks Evans.

This prompted him to form the Stem Cell Foundation, a charity designed to plug the gap between academic research and mid-stage clinical trials. "In three years we should have 10 to 15 projects approaching or in the clinic. With the usual attrition rate this will translate into two or three successes, and we will then get [private investment] money flowing in", says Evans. "The foundation is the catalyst—we will create a phenomenon in stem cells".

Evans is keen to get the foundation up and running before the money starts flowing from California's Proposition 71 and other US state funding schemes for stem cells and thus prompts a brain drain of researchers from the UK to the US.

But the fact that California and other states are raising their own budgets for stem cell research highlights yet another hurdle in the way of its commercialisation. Uniquely, for a medical product, it is unclear whether it will be possible to get a single regulatory approval to sell a stem cell therapy across the US or whether the states with bans on embryonic stem cell research will ban products based on them also.

The situation is no better in Europe, where there is a patchwork of different regulation, most of it militating against embryonic stem cell research.

Cathy Prescott, science director at Avlar BioVentures in Cambridge, UK, says: "The major issue is on the regulatory side of things at the moment. National rules are applying in Europe, and in the US different states have taken a different stance, and therefore there is a fragmented marketplace".

Most biotechnology companies rely on doing deals with big pharmaceutical companies to get their products through the later stages of clinical trials and on to the market.

"The market fragmentation is making stem cells a very, very difficult business model for big pharma", says Prescott. "If biotechs haven't got partners, how can they take it forward"?

No doubt VCs are daunted by the ethical and regulatory baggage surrounding stem cells. Several prominent firms in North America and Europe did not wish to be interviewed for this article. Others were prepared to discuss the scientific challenges but not the baggage.

Proposition 71 will change attitudes, believes Burrill of Burrill & Company: "At present, stem cell science is tainted. Proposition 71 will legitimise a lot of research in the US, which under federal guidelines is perceived to be not investible".

Nuala Moran is UK correspondent for BioWorld.

"Businesses need to be more sophisticated about how they control risk. You wouldn't back a conventional science company that had only one product".
—BRIAN KERR

NOMURA PHASE4 VENTURES (*top*); SCOTTISH EQUITY PARTNERS (*bottom*)

The Search for Cells That Heal

Ian Wilmut, creator of Dolly the cloned sheep, urges looking past the controversies to the ultimate payoff

Extraordinary opportunities to study and to treat human diseases are provided by the recently acquired ability to derive stem cells from human embryos. Because these cells form all of the tissues that make up an adult, they afford a chance to study normal human development in the laboratory, to define the abnormalities associated with inherited disease and, in time, perhaps to treat diseases, many of which have no effective treatment at present.

Consider just three situations among many. Cells derived from embryo cells could be used to repair spinal cord injury. It is far from clear exactly what type of cell should be used, how many cells are needed or where they should be placed. Nevertheless, speedy treatment might provide real benefit.

Cells from cloned embryos will reveal the molecular mechanisms that cause inherited diseases such as amyotrophic lateral sclerosis (known as motor neurone disease in some countries). This will allow us to study the disease process in minute detail for the first time and, more important, to screen thousands of compounds that might potentially arrest or even reverse the degeneration.

Finally, genetic diseases may eventually be corrected in children. Imagine a child who has no immune response to infection because of an error in a specific gene. The error could be corrected in cells derived from a cloned embryo, which might then be converted to bone marrow cells that provide the absent immune response. The corrective marrow cells could then be returned to the child.

Clearly, success with embryonic stem cells will depend upon detailed research, and it will take several years, perhaps decades, to bring these ideas to the clinic. Over time, embryo-derived stem cells will revolutionise many aspects of medicine. And yet society hesitates.

In discussing stem cell research, investigators face several critical issues. To some people the idea of producing and using a human embryo is deeply offensive, and these sincerely held views must be recognised. Yet many others do not share these qualms. The early embryo from which stem cells are derived is a ball of cells smaller than a grain of sand. While it has the potential to become a person, it lacks the fundamental human characteristics of being conscious and aware.

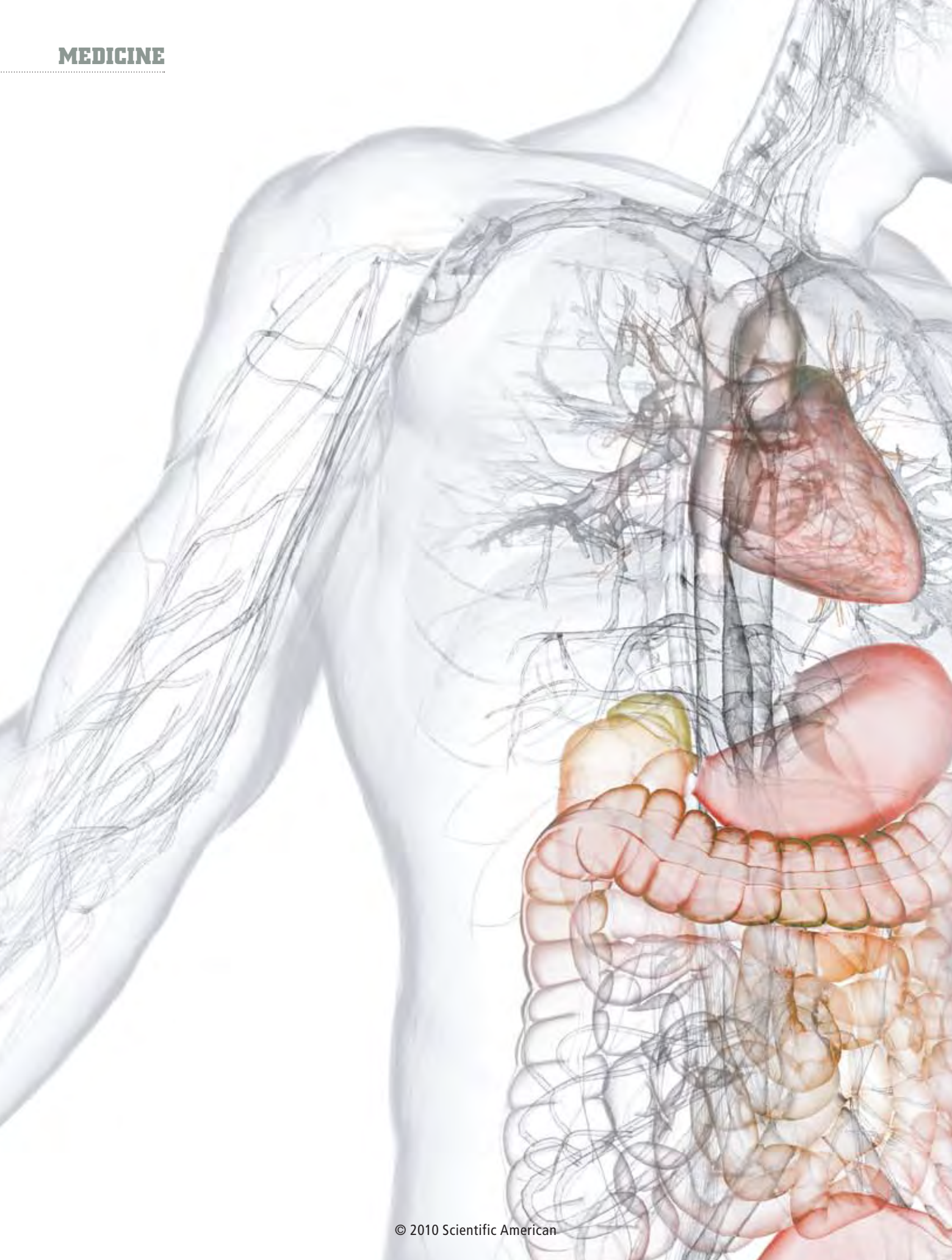
An urgent need exists for an informed debate about what we consider to be critical human characteristics, just as there was an equivalent debate about the end of life when decisions were first made to remove organs from accident victims who were brain-dead but had healthy organs.

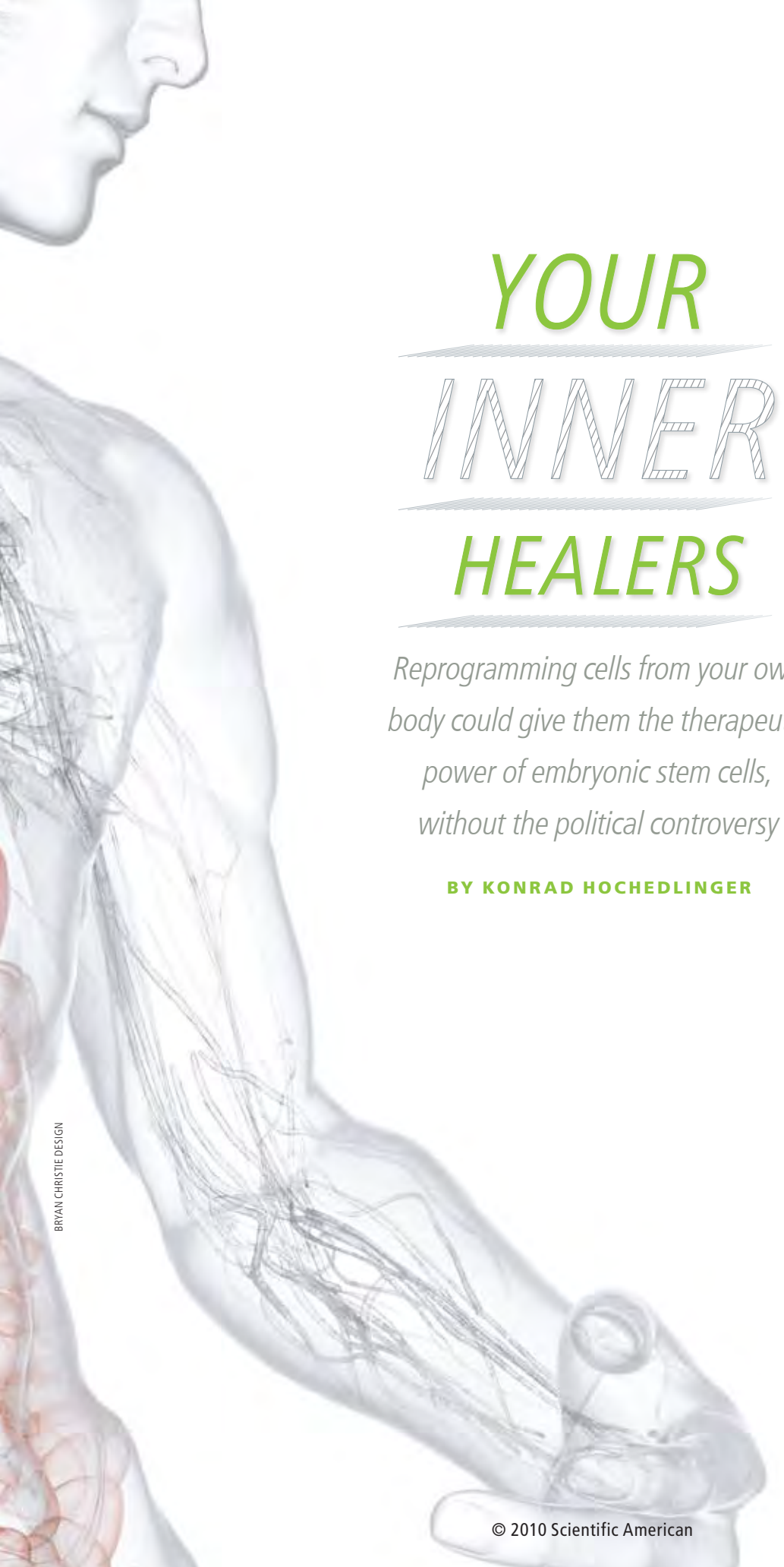
The potential benefits of stem cells should inspire optimism, but this must also be tempered with the frank admission that we still have far, far more to learn about embryonic stem cells. Unfortunately, the time required for the development of clinical treatments will be beyond that usually accepted by venture-capital investors, and it seems likely that a partnership will be needed between government sources of funds and private capital.

Anyone who knows or has cared for a person with an inherited or degenerative disease knows only too well the great need for new treatments. We should be excited by the opportunity rather than afraid.

Ian Wilmut is Chair in Reproductive Science at the University of Edinburgh in Scotland and a visiting scientist at the Roslin Institute.

COLIN McPHERSON *Corbis*





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.

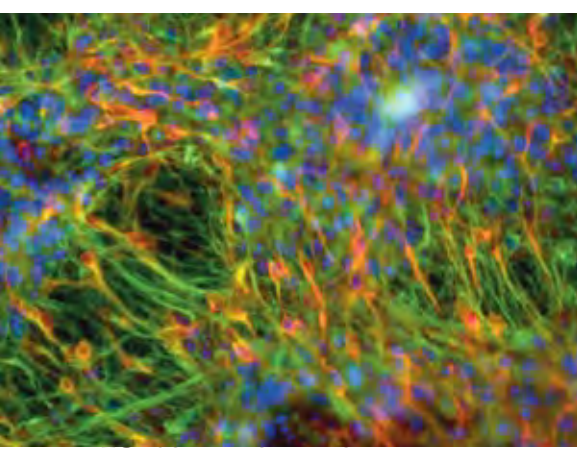
Throughout human history people have dreamed of finding a Fountain of Youth to escape the consequences of aging and disease.

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-



THERAPEUTIC PROMISE

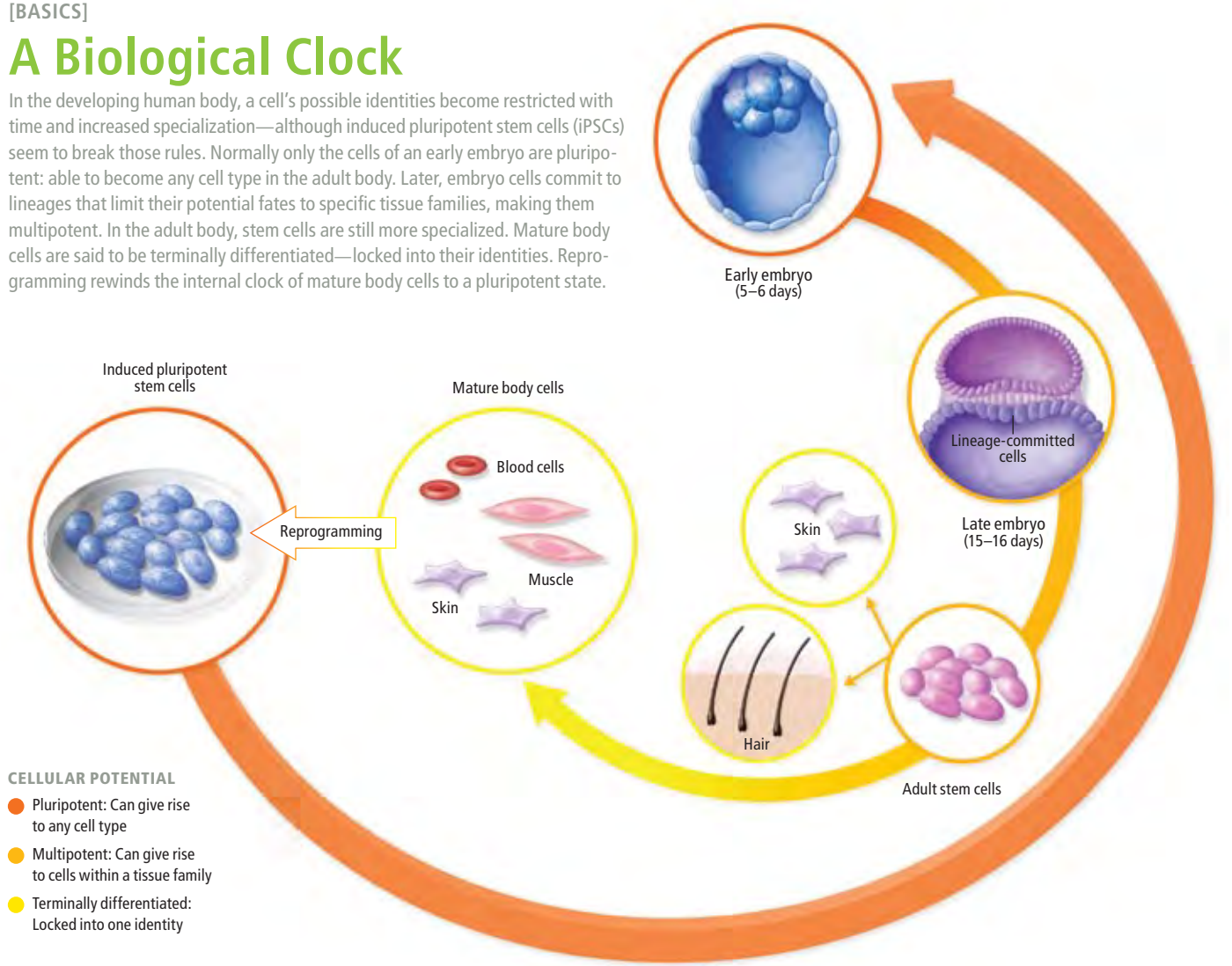
Neurons (*left*) were generated from induced pluripotent cells that were made from the skin cells of patients with Parkinson's disease. With the ability to take a mature body cell and convert it to an embryonic state, then into any desired tissue type, scientists will be able to study how a variety of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

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

[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.

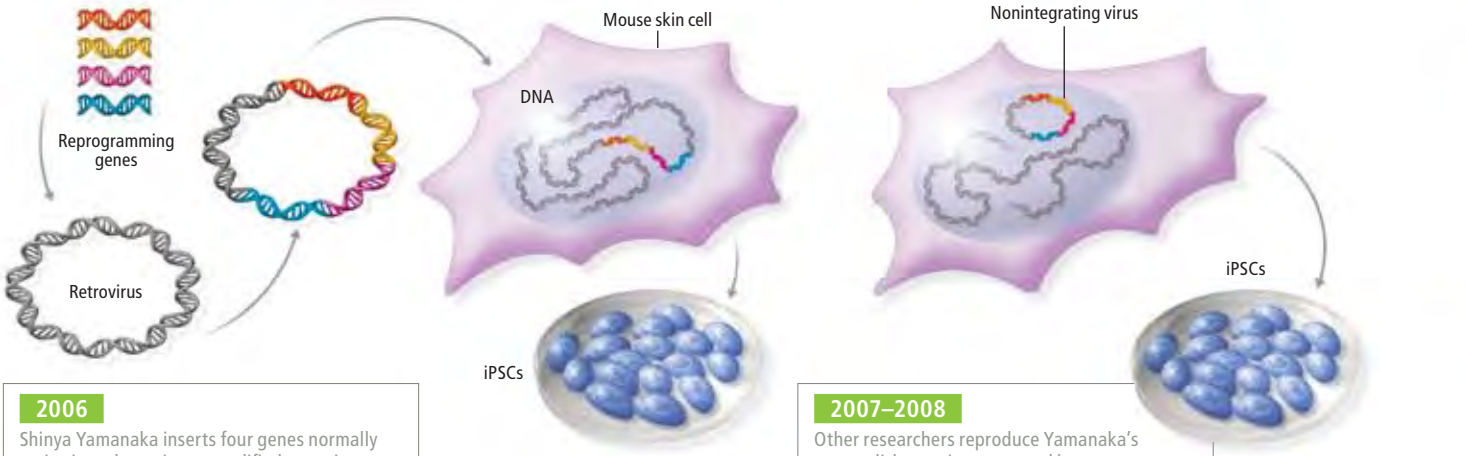
Yamanaka and his group went around this impasse by taking a novel approach to turning

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

[MILESTONES]

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

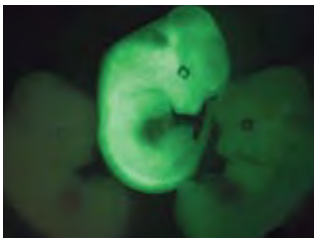
Shinya Yamanaka inserts four genes normally active in embryos into a modified retrovirus, which he then injects into mouse skin cells. The virus inserts the genes into the mouse DNA, and the genes then begin reprogramming the skin cells into induced pluripotent stem cells (iPSCs).

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

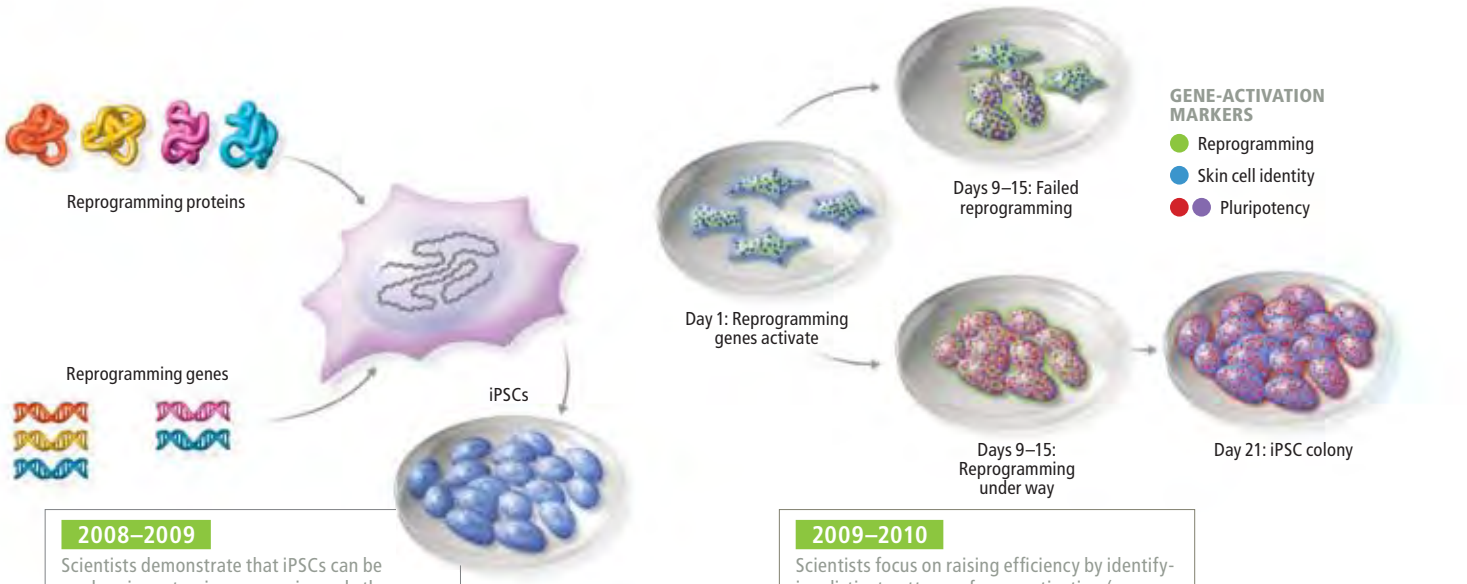
Gold-standard laboratory tests to determine whether stem cells are truly pluripotent aim to demonstrate that the cells can give rise to any tissue type in the body. When injected into an early mouse embryo, for example, fluorescently marked pluripotent cells should integrate throughout the body of the 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*)



2008–2009

Scientists demonstrate that iPSCs can be made using retroviruses carrying only three of the original Yamanaka reprogramming genes, then only two, or just by introducing the proteins encoded by the four reprogramming genes directly into cells.

2009–2010

Scientists focus on raising efficiency by identifying distinct patterns of gene activation (revealed by fluorescent markers) characterizing cells that will successfully convert to iPSCs. Skin cell identity and reprogramming-gene markers give way to pluripotency markers.

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]

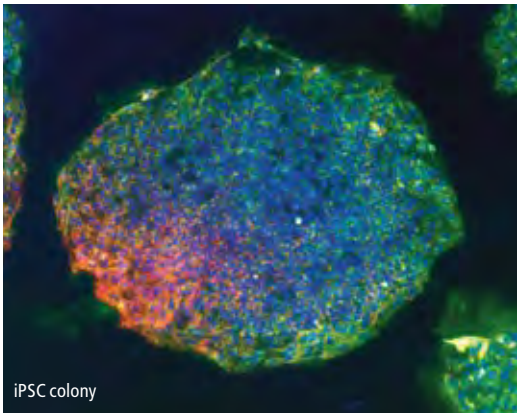
Konrad Hochedlinger is associate professor of stem cell and regenerative biology at Harvard University and a faculty member of the Harvard Stem Cell Institute and the Howard Hughes Medical Institute. In his laboratory at Massachusetts General Hospital, he works toward understanding the biology of stem 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.

COURTESY OF CELLULAR DYNAMICS INTERNATIONAL, INC.

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.

Epigenetic Reprogramming and Induced Pluripotency. Konrad Hochedlinger and Kathrin Plath in *Development,* Vol. 136, No. 4, pages 509–523; February 15, 2009.

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

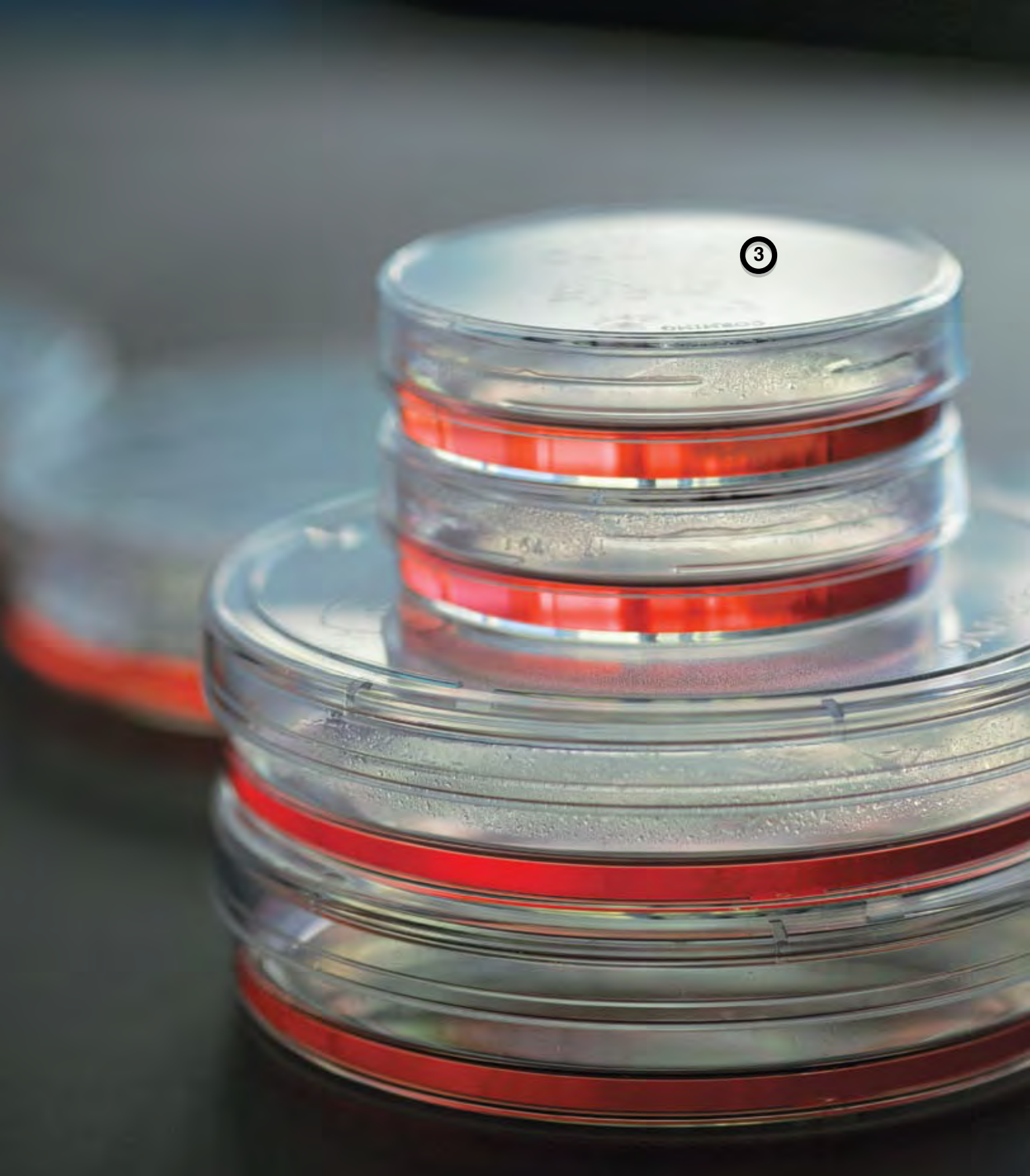
Although iPSCs clearly circumvent some of the ethical and legal controversies surrounding embryonic cells, their pluripotency has yet to be completely understood or controlled, and embryonic stem cells therefore remain the gold standard for any pluripotent cell type.

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. ■



After stem cells grow for 30 days in culture medium (*red*), they become specialized tissue that can be used to model different diseases.

MEDICINE

diseases in a dish

A creative use of stem cells made from adult tissues may hasten drug development for debilitating diseases

By Stephen S. Hall

Photographs by Grant Delin

Stephen S. Hall described the early history of stem cell research in the award-winning *Merchants of Immortality* (Houghton Mifflin, 2003). His most recent book, *Wisdom: From Philosophy to Neuroscience* (Vintage), will be issued in paperback in March.

ON JUNE 26, 2007, WENDY CHUNG, director of clinical genetics at Columbia University, drove to the New York City borough of Queens with a delicate request for the Croatian matriarchs of a star-crossed family. She asked the two sisters, one 82 and the other 89, if they would donate some of their skin cells for an ambitious, highly uncertain experiment that, if it succeeded, promised a double payoff. One, it might accelerate the search for treatments for the incurable disease that ran in their family. Two, it might establish a valuable new use for stem cells: unspecialized cells able to give rise to many different kinds of cells in the body. "We had a very nice lunch and literally went back to the house and took the biopsies," Chung remembers. As they sat around the dining-room table, the elderly sisters were "very happy sticking out their arms," recalls the daughter of the 82-year-old woman. The younger sister told Chung: "I get it. Go right ahead."

The sisters suffered from amyotrophic lateral sclerosis (ALS), a degenerative and slowly paralyzing nerve disorder that is also known as Lou Gehrig's disease, after the Yankee slugger who was told he had it in 1939 and died two years later. The 89-year-old showed few signs of the disease, whereas her 82-year-old sister had trouble walking and swallowing.

Although most cases of ALS are not hereditary, the disorder has struck multiple members of this particular family. Affected members inherited a mutation that has been linked to a more slowly progressing form of the disease than the one that attacks most other people with the condition. Chung had been tracking the disorder across several generations of the family in Europe and the U.S. "Lou Gehrig's disease is not a pretty way to die," she says. "Every time family members would get together at funerals, people in the younger generation would be looking around and asking, 'Am I going to be next?'"

It took Chung just a couple minutes to perform the actual "punch biopsy"—two quick nips of flesh, each three millimeters in diameter, from the inner arm. Eventually the sisters' cells, along with skin samples from dozens of other ALS patients and healthy volunteers who similarly donated bits of tissue, were chemically induced to become a form of stem cell known as an induced pluripotent stem cell and were then reprogrammed to become nerve cells. Specifically, they were induced to become motor neurons, the nerve cells that directly or indirectly control the muscles of the body and are adversely affected by ALS. The resulting tissue cultures exhibited the same molecular defects that gave rise to ALS in their human donors. In other words, the investigators had, to an astonishing extent, re-created the disease in a petri dish.

With these cells in hand, they could begin to study exactly what goes wrong in the nerve cells of ALS patients and could start to screen potential drugs for useful effects on the diseased cells. This use of stem cells is new and contrasts with so far disappointingly slow progress in efforts to use stem cells as therapies. If successful, the disease-in-a-dish concept could speed up researchers' understanding of many different diseases and lead to faster, more efficient screening of potential drug therapies, because scientists can test potential drugs in these custom-made cultures for both therapeutic efficacy and toxicity. In addition to the ALS work, the induced stem cells are currently being used experimentally to model dozens of illnesses, including sickle cell anemia, many other blood disorders and Parkinson's disease. Researchers in Germany, for example, have created cardiac cells that beat irregularly, mimicking various heart arrhythmias. Pharmaceutical companies, long wary of stem cell science as a commercial enterprise, are starting to show greater interest because the disease-in-a-dish approach complements the traditional strengths of industrial drug discovery.

The first fruit of the ALS experiment was published in 2008. As in most cases of innovation, success depended not only on the soundness of the idea but on the right mix of people pursuing it. In this case, the cast of characters, in addition to Chung, included Lee L. Rubin, a refugee from the biotech industry who

IN BRIEF

Still waiting: Stem cells from embryos hold promise for treating incurable conditions; however, investigators have not so far made much progress in deriving therapies from stem cells.

A new idea: Rather than focusing on treatments, a few researchers think stem cells are better suited—for now—to help screen for drugs and to investigate how different diseases damage the body.

Creative approach: Until recently, the stem cells needed to pursue this idea were made using embryos. But in 2007 scientists managed to reprogram adult human cells into stem cells.

Customized stem cells: Researchers are using these reprogrammed cells to re-create various diseases in a petri dish. Then they can test potential drugs against the refashioned tissue samples.

became head of translational medicine at the Harvard Stem Cell Institute, and Kevin C. Eggan, a tireless young stem cell scientist from Harvard, who was collaborating with Christopher E. Henderson and other motor neuron experts at Columbia.

A NEW ROLE FOR STEM CELLS

THE STEM CELLS used in these studies should not be confused with embryonic stem cells—the kind derived from early embryos. A dozen years ago James A. Thomson and his colleagues at the University of Wisconsin–Madison electrified the world with the news that they had created human embryonic stem cells in a lab for the first time. These primordial cells had the biological endurance to renew themselves forever and the versatility to turn into any cell type in the body. The possibility of using stem cells to create made-to-order transplants for everything from Parkinson's to diabetes tantalized doctors, researchers, the public at large and, most of all, patients with incurable conditions.

But two harsh realities awaited. First, a loud public debate over the ethics of stem cell science politicized the science and slowed research; the technology posed moral questions because human embryos had to be destroyed to harvest the embryonic stem cells. That debate culminated in President George W. Bush's announcement in August 2001 that the National Institutes of Health would restrict funding support to research using only a few existing embryonic stem cell lines, which effectively impeded the generation of additional stem cells, including the disease-specific cell lines. In response, prominent research groups at Harvard, Columbia and Stanford universities, along with patient advocacy groups such as Project ALS and the New York Stem Cell Foundation, created separate, "nonpresidential" labs to pursue research with private funding. In 2009 the Obama administration relaxed the rules governing stem cell research, but a federal court ruling in 2010 banned grant support from the National Institutes of Health once again and plunged the field into scientific uncertainty and funding chaos.

The second problem was scientific. As Valerie Estess, scientific director of Project ALS, recalls it, there was a mad rush to test the idea that specialized cells derived from stem cells could simply be transplanted into sick people (or animals) as cellular therapies to cure a host of diseases. "The big dream," she explains, "was to derive motor neurons from stem cells, and then you would put them in the brain or spinal cord, and the patients would just get up and start dancing the Watusi." But it did not work out that way in repeated animal experiments. "From beginning to end," Estess says, "these experiments were failures."

In 2002 Thomas M. Jessell, Hynek Wichterle and their team at Columbia published a landmark paper in the journal *Cell,* spelling out the ingredients and procedure for nudging embryonic stem cells down a biological pathway to form motor neurons. One researcher who saw in that work promise for a different use of stem cells was Rubin. Elfin and enthusiastic, Rubin had trained in neuroscience and served as research and chief scientific officer of a Massachusetts biotech company called Curis. He realized that creating a disease in a dish offered a potentially revolutionary way to discover drugs. And unlike a lot of academic scientists, he knew something about drug discovery. During a previous stint in biotech, he worked on a molecule that ultimately became the billion-dollar multiple sclerosis drug Tysabri.

After hearing the results of Jessell and Wichterle's research, Rubin drafted a business plan for a new kind of stem cell institute, "one that focused," he says, "not on cell therapy—which all stem cell biologists were interested in—but on using stem cells to discover drugs." At the time, venture capitalists wanted nothing to do with the idea. So Rubin nursed the idea along at Curis, working on spinal muscular atrophy, a childhood motor neuron disease that has a similar pathology to ALS. When Curis decided to drop the project in 2006, he quit biotech and moved to the Harvard Stem Cell Institute to pursue the disease-in-a-dish idea.

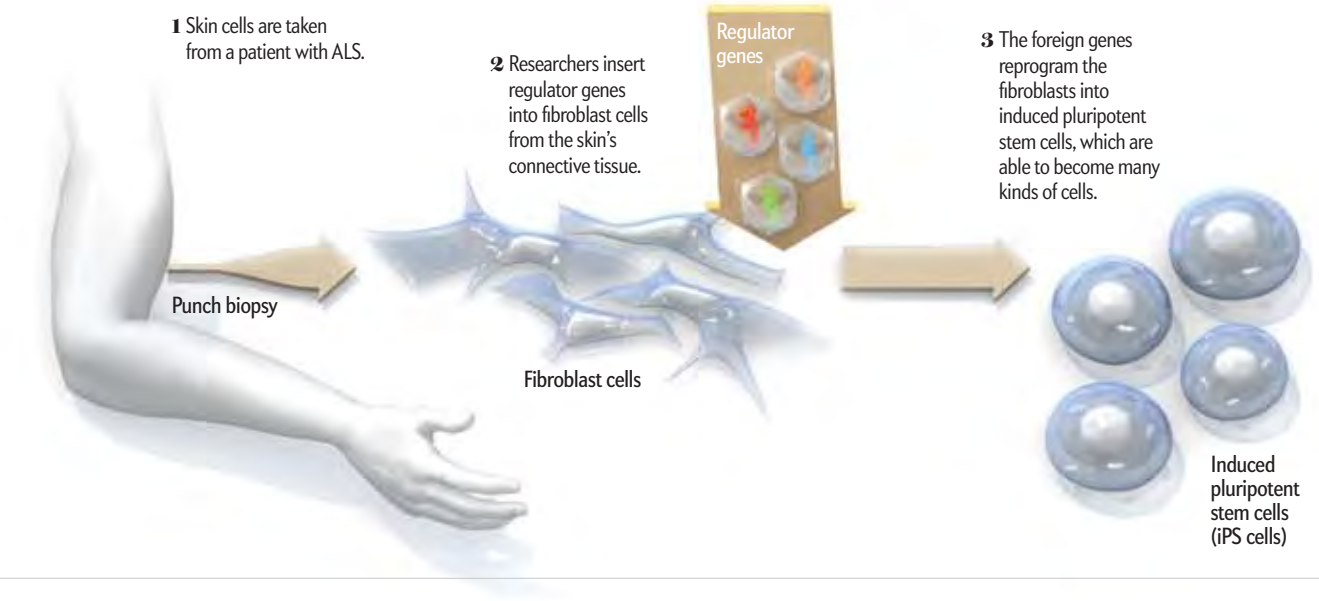
Shortly afterward, a Japanese biologist named Shinya Yamanaka disclosed a technique that would ultimately transform both stem cell biology and stem cell politics. At a scientific meeting at Whistler, B.C., in March 2006, the Kyoto University scientist described a procedure by which biologists could take ordinary adult mammalian cells and "reprogram" them. In essence, Yamanaka had biochemically reset the adult cells back to an embryoniclike or stemlike state without needing to use or destroy an embryo. He called the cells "induced pluripotent stem cells," or iPS cells. A year later both Yamanaka and Wisconsin's Thomson separately reported that they had created iPS cells from human tissue [see "Your Inner Healers," by Konrad Hochedlinger; SCIENTIFIC AMERICAN, May 2010].

One of the people sitting in the audience that day in Whistler

Cold storage: Biopsies and stem cells are preserved in liquid nitrogen.

New Uses for Old Skin

Using techniques pioneered in Japan, researchers from Harvard and Columbia universities extract skin tissue from adults (*below*), isolate specialized cells called fibroblasts from the sample, then gently coax them with genes and chemicals to become nerve cells.



was Eggan, who was a cellular reprogramming expert at Harvard. In fact, he had already embarked on his own version of the disease-in-a-dish idea, launching several projects to take an adult cell and biochemically coax it back into an embryolike state, allow it to replicate, and harvest stem cells from the resulting colony. He was trying to make embryolike cells the "old-fashioned" way, however, by applying the same cloning technique that produced Dolly the sheep. Eggan would take the nucleus out of an adult cell, such as a skin cell, and implant it into an unfertilized egg whose own nucleus had been removed. Cloning, however, was terribly inefficient and also terribly controversial if you planned to reprogram human cells—not least because you had to find women willing to donate their egg cells for the procedure.

Using Yamanaka's approach, however, Eggan and his team finally got the iPS technique to work in a test run with human cells in the summer of 2007. Everything else was already in place to try the disease-in-a-dish concept. Chung and her Columbia colleagues, for example, had collected cells from the two Croatian sisters and other ALS patients in anticipation that they would be used in Eggan's cloning experiments. With private funding, Project ALS had created a special laboratory near Columbia where researchers had been stockpiling cell lines from patients (including the elderly sisters) for months. Suddenly, the iPS approach offered a better chance of success. "That was complete kismet, that we had begun to collect human skin cells with a very different experiment in mind," says Estess of Project ALS.

The headliner among all those first ALS cell lines was the one from the younger, sicker Croatian sister, identified as patient A29. The skin cells of both sisters were successfully reprogrammed into nerve cells, but the age and degree of illness in patient A29 demonstrated that the iPS technique could be used to create cells that reflected a serious, lifelong disease. "We chose those samples because those were the oldest people in our study," Eggan says. "We wanted to prove the point that you could reprogram cells even from a very, very, very, very old person who'd been sick for some length of time. They were a special case."

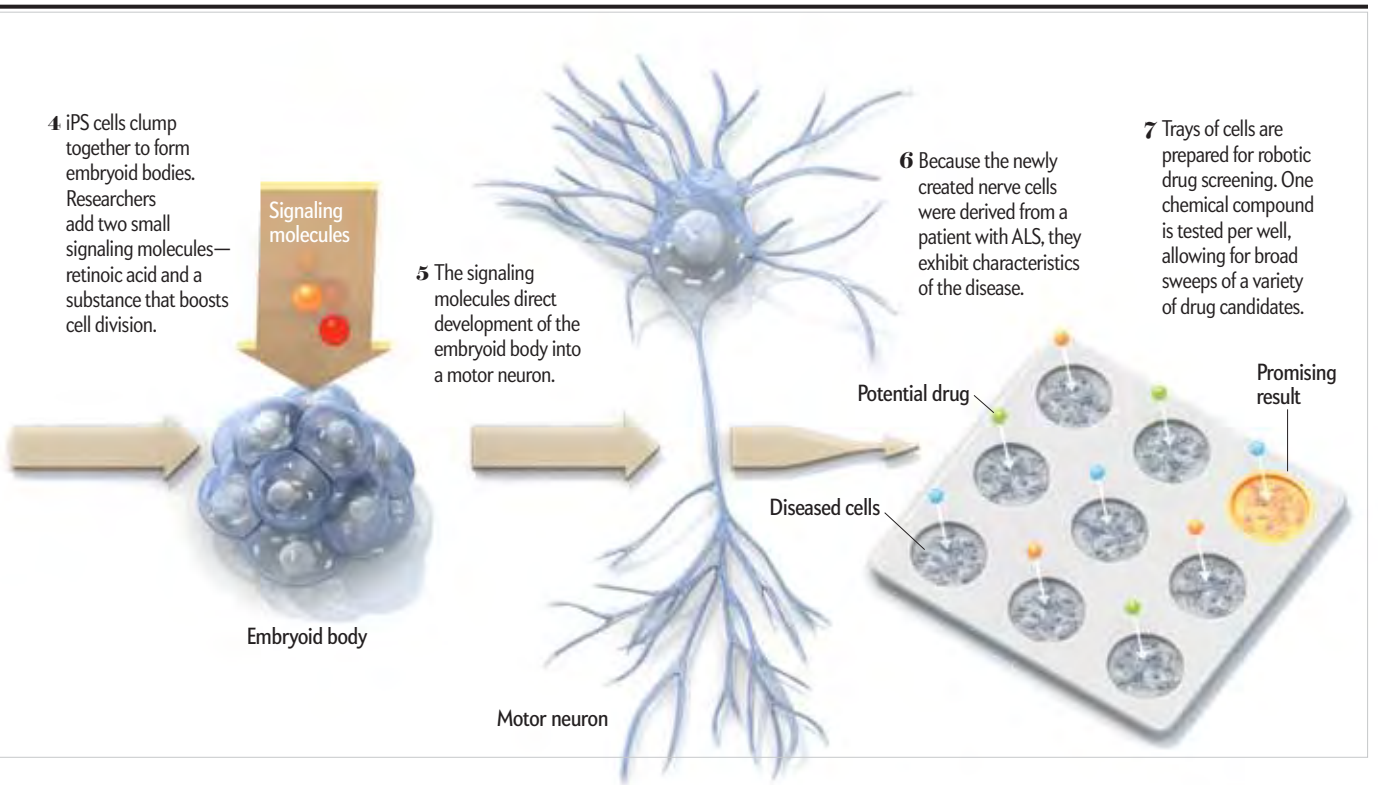
The results appeared in the August 29, 2008, issue of *Science* and were hailed in the press as a scientific milestone. The idea of using stem cells to create a disease in a dish promised experimental access to cells that were otherwise difficult or impossible to obtain—the motor neurons characteristic of ALS and spinal muscular atrophy, brain cells in many neurodegenerative disorders, and pancreatic cells typical of juvenile diabetes.

MADE-TO-ORDER STEM CELLS

IN THE PAST TWO YEARS the Columbia-Harvard collaboration has produced no fewer than 30 ALS-specific human cell lines, with more on the way. Many of these cell lines capture unique mutations found in people with unusually severe cases of ALS. More important, the disease-in-a-dish approach is beginning to deliver on its potential, providing insights into the nature of motor neuron disease. Using cells from the two sisters, for example, researchers have identified molecular pathways that seem to be involved in the death of motor neurons, which occurs when these cells are poisoned by another class of neurons known as astrocytes. With both motor neurons and astrocytes in a dish, scientists are now searching for potential therapeutic compounds that can either block the toxic activity of astrocytes or enhance the survival of motor neurons.

In January 2010, for example, researchers at the Project ALS lab began a preliminary screen of about 2,000 compounds in ALS motor neurons from humans, looking to see if any of the molecules would prolong the survival of nerve cells that contain the mutated ALS gene. This initial pilot program reflects a novel approach to drug screening: the ALS researchers began by testing compounds that have already been approved by the Food

Illustration by Bryan Christie



and Drug Administration for other illnesses. The hope is that researchers might get lucky and find a molecule, already tested and proved safe in humans, that could be rapidly repurposed for motor neuron disease. Pursuing a parallel track at Harvard, Rubin has identified almost two dozen small molecules that interact with one of the newly identified pathways and enhance the survival of motor neurons. The Spinal Muscular Atrophy Foundation is currently testing one of the molecules in an animal model of spinal muscular atrophy.

Perhaps an equally telling indicator that iPS cells offer a promising approach to drug discovery is the fact that Rubin is no longer banging his head against the door of pharmaceutical companies. Since the Columbia and Harvard researchers established the principle of a disease in a dish—that neurons with the genetic makeup of those in a diseased person can be produced—with patient A 29 in the summer of 2008, pharmaceutical companies have been banging on Rubin's door. Without naming specific companies for confidentiality reasons, he says, "I would say that of the major pharmaceutical companies, all of them have become interested in this approach now." The excitement has spilled over into biotech: many of the researchers in the motor neuron disease-in-a-dish story, including Eggan and Rubin, have become involved in a California-based biotechnology company called iPierian, which is one of several startups, including Cellular Dynamics International and Fate Therapeutics, that are adapting iPS technology for drug discovery.

MORE ON IPS CELLS
ScientificAmerican.com/mar2011/ips

Meanwhile more and more stem cell researchers are pursuing the disease-in-a-dish concept. Shortly after the ALS publication in 2008, a separate group of researchers at the Harvard Stem Cell Institute reported using the iPS technique to create disease-in-a-dish cells from patients with juvenile diabetes, Parkinson's and other disorders. And in late 2008 researchers at Wisconsin, led by Clive N. Svendsen (who has since moved to Cedars-Sinai Medical Center in Los Angeles), created motor neurons in a dish from a patient with spinal muscular atrophy.

When I asked researchers at Columbia and Harvard if the two Croatian sisters were aware of the research that grew out of their donated cells, no one seemed to know at first. But I eventually learned that the sisters are still alive, according to the daughter of patient A29, who agreed to speak as long as her name and those of family members remained anonymous. The older sister, now 93, remains essentially free of symptoms of ALS; indeed, according to her niece, she still "lives by herself, walks everywhere, shops, cooks, sweeps and cleans." The younger sister, patient A29, turned 85 last June; despite her ALS, she can move "slowly and weakly" and is "grateful" to have had the opportunity to help.

Still, the family's cruel burden never seems far away and underscores the urgency felt by those who might benefit from the new stem cell approach to finding drugs. "I am relatively young," says patient A29's daughter, who herself was diagnosed with ALS in 2002. "We are afraid that the onset of the disease is becoming earlier as the generations go along. We feel a little like"—she pauses as she speaks, to gather herself and her inevitably grim thoughts—"it's a race against time. I myself have a teenage daughter, and it just weighs so heavily on the mind and heart." SA

MORE TO EXPLORE

Induced Pluripotent Stem Cells Generated from Patients with ALS Can Be Differentiated into Motor Neurons. John T. Dimos et al. in *Science,* Vol. 321, pages 1218–1221; August 29, 2008.
Study Says Brain Trauma Can Mimic A.L.S. Alan Schwarz in *New York Times,* August 17, 2010.
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PANDORA'S BABY

In vitro fertilization was once considered by some to be a threat to our very humanity. Cloning inspires similar fears

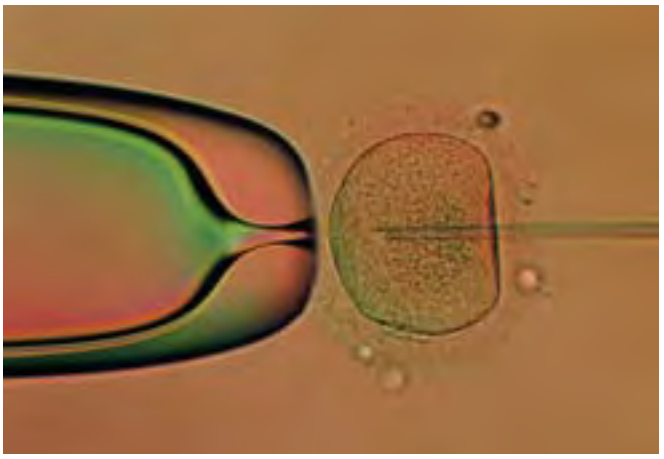
BY ROBIN MARANTZ HENIG

On July 25, a once unique person will turn 25. This nursery school aide in the west of England seems like an average young woman, a quiet, shy blonde who enjoys an occasional round of darts at the neighborhood pub. But Louise Brown's birth was greeted by newspaper headlines calling her the "baby of the century." Brown was the world's first test tube baby.

Today people may remember Brown's name, or that she was British, or that her doctors, Steptoe and Edwards, sounded vaguely like a vaudeville act. But the past quarter of a century has dimmed the memory of one of the most important aspects of her arrival: many people were horrified by it. Even some scientists feared that Patrick Steptoe and Robert Edwards might have brewed pestilence in a petri dish. Would the child be normal, or would the laboratory manipulations leave dreadful genetic derangements? Would she be psychologically scarred by the knowledge of how bizarrely she had been created? And was she a harbinger of a race of unnatural beings who might eventually be fashioned specifically as a means to nefarious ends?

Now that in vitro fertilization (IVF) has led to the birth of an estimated one million babies worldwide, these fears and speculations may seem quaint and even absurd. But the same concerns once raised about IVF are being voiced, sometimes almost verbatim, about human cloning. Will cloning go the way of IVF, morphing from the monstrous to the mundane? And if human cloning, as well as other genetic interventions on the embryo, does someday become as commonplace as test tube baby–making, is that to be feared—or embraced? The lessons that have been

RYSZARD HOROWITZ *Corbis*



MICRONEEDLE INJECTS a sperm's package of DNA directly into a human egg, thereby achieving in vitro fertilization (*left*). The first human being born as a result of IVF, Louise Brown was 14 months old when she frolicked on the set of the *Donahue* television program (*right*). With her was Vanderbilt University IVF researcher Pierre Soupart, who predicted that "by the time Louise is 15, there will be so many others it won't be remarkable anymore."

learned from the IVF experience can illuminate the next decisions to be made.

Then and Now

AS IVF MOVED FROM the hypothetical to the actual, some considered it to be nothing more than scientists showing off: "The development of test tube babies," one critic remarked, "can be compared to the perfecting of wing transplants so that pigs might fly." But others thought of IVF as a perilous insult to nature. The British magazine *Nova* ran a cover story in the spring of 1972 suggesting that test tube babies were "the biggest threat since the atom bomb" and demanding that the public rein in the unpredictable scientists. "If today we do not accept the responsibility for directing the biologist," the *Nova* editors wrote, "tomorrow we may pay a bitter price—the loss of free choice and, with it, our humanity. We don't have much time left."

A prominent early enemy of IVF was Leon Kass, a biologist at the University of Chicago who took a professional interest in the emerging field of bioethics. If society allowed IVF to proceed, he wrote shortly after Louise Brown's birth, some enormous issues were at stake: "the idea of the humanness of our human life and the meaning of our embodiment, our sexual being, and our relation to ancestors and descendants."

Now read Kass, a leading detractor of every new form of reproductive technology for the past 30 years, in 2003: "[Cloning] threatens the dignity of human procreation, giving one generation unprecedented genetic control over the next," he wrote in the *New York Times*. "It is the first step toward a eugenic world in which children become objects of manipulation and products of will." Such commentary coming from Kass is particularly noteworthy because of his unique position: for the past two years he has been the head of President George W. Bush's Council on Bioethics, whose first task was to offer advice on how to regulate human cloning.

Of course, IVF did not wind up creating legions of less than human children, nor did it play a role in the disintegration of the nuclear family, consequences that people like Kass feared. And so many newer, more advanced methods of assisted reproduction have been introduced in the past decade that the "basic IVF" that produced Louise Brown now seems positively routine. One early prediction, however, did turn out to contain more than a kernel of truth. In the 1970s critics cautioned that IVF would set us tumbling down the proverbial slippery slope toward more sophisticated and, to some, objectionable forms of reproductive technology—and that once we opened the floodgates by allowing human eggs to be fertilized in the laboratory, there would be no stopping our descent.

If you consider all the techniques that might soon be available to manipulate a developing embryo, it could appear that the IVF naysayers were correct in their assessment of the slipperiness of the slope. After all, none of the genetic interventions now being debated—prenatal genetic diagnosis, gene insertions in sex cells or embryos to correct disease, the creation of new embryonic stem cell lines and, the elephant in the living room, cloning—would even be potentialities had scientists not first learned how to fertilize human eggs in a laboratory dish.

But does the existence of a such a slippery slope mean that present reproductive technology research will lead inevitably to developments that some find odious, such as embryos for tissue harvesting, or the even more abhorrent manufacture of human-nonhuman hybrids and human clones? Many people clearly fear so, which explains the current U.S. efforts to

Overview/*In Vitro Veritas*

- Many arguments against in vitro fertilization in the past and cloning today emphasize a vague threat to the very nature of humanity.
- Critics of IVF attempted to keep the federal government from supporting the research and thus ironically allowed it to flourish with little oversight.
- Because of the lack of oversight, it is only in the past few years that the increased rate of birth defects and low birth weight related to IVF have come to light.

PASCAL GOETGHELUCK *Science Photo Library* (*left*); RAY FOLI *Corbis-Bettmann* (*right*)

MEMBERS of the Christian Defense Coalition and the National Clergy Council protest Advanced Cell Technologies's human cloning research outside the biotechnology firm's headquarters in Worcester, Mass., on November 30, 2001. Similar protests against IVF occurred in the 1970s.

WILLIAM PLOWMAN *Getty*

curtail scientists' ability to manipulate embryos even before the work gets under way. But those efforts raise the question of whether science that has profound moral and ethical implications should simply never be done. Or should such science proceed, with careful attention paid to the early evolution of certain areas of research so that society can make informed decisions about whether regulation is needed?

IVF Unbound

THE FRENZY TO TRY to regulate or even outlaw cloning is in part a deliberate attempt not to let it go the way of IVF, which has been a hodgepodge of unregulated activities with no governmental or ethical oversight and no scientific coordination. Ironically, the reason IVF became so ubiquitous and uncontrolled in the U.S. was that its opponents, particularly antiabortion activists, were trying to stop it completely. Antiabortion activists' primary objection to IVF was that it involved the creation of extra embryos that would ultimately be unceremoniously destroyed—a genocide worse than at any abortion clinic, they believed. Accordingly, they thought that their best strategy would be to keep the federal government from financing IVF research.

A succession of presidential commissions starting in 1973 debated the ethics of IVF but failed to clarify matters. Some of the commissions got so bogged down in abortion politics that they never managed to hold a single meeting. Others concluded that IVF research was ethically acceptable as long as scientists honored the embryo's unique status as a "potential human life," a statement rather than a practical guideline. In 1974 the government banned federal funding for fetal research. It also forbade funding for research on the human embryo (defined as a fetus less than eight weeks old), which includes IVF. In 1993 President Bill Clinton signed the NIH Revitalization Act, which allowed federal funding of IVF research. (In 1996, however, Congress again banned embryo research.) The bottom line is that despite a series of recommendations from federal bioethics panels stating that taxpayer support of IVF research would be acceptable with certain safeguards in place, the government has never sponsored a single research grant for human IVF.

This lack of government involvement—which would also have served to direct the course of IVF research—led to a funding vacuum, into which rushed entrepreneurial scientists supported by private money. These free agents did essentially whatever they wanted and whatever the market would bear, turning IVF into a cowboy science driven by the marketplace and undertaken without guidance. The profession attempted to regulate itself—in 1986, for example, the American Fertility Society issued ethical and clinical guidelines for its members—but voluntary oversight was only sporadically effective. The quality of clinics, of which there were more than 160 by 1990, remained spotty, and those seeking IVF had little in the way of objective information to help them choose the best ones.

Today, in what appears to be an effort to avoid the mistakes made with IVF, the federal government is actively involved in regulating cloning. With the announcement in 1997 of the birth of Dolly, the first mammal cloned from an adult cell, President Clinton established mechanisms, which remain in place, to prohibit such activities in humans. Congress has made several attempts to outlaw human cloning, most recently with a bill that would make any form of human cloning punishable by a $1-million fine and up to 10 years in prison. (The House of Representatives passed this bill this past winter, but the Senate has yet to debate it.) Politicians thus lumped together two types of cloning that scientists have tried to keep separate: "therapeutic," or "research," cloning, designed to produce embryonic stem cells that might eventually mature into specialized human tissues to treat degenerative diseases; and "reproductive" cloning, undertaken specifically to bring forth a cloned human being. A second bill now

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From Outrage to Approval

THE STORY of Doris Del-Zio demonstrates the ironies resulting from society's changing attitude toward IVF in the 1970s. After years of failure to conceive a child, Del-Zio and her husband turned to Landrum Shettles of what is now known as the Columbia Presbyterian Medical Center. In the fall of 1973 Shettles prepared to attempt a hasty IVF procedure on the couple. The operation was abruptly terminated by Shettles's superior, Raymond Vande Wiele, who was outraged at Shettles's audacity and who questioned the medical ethics of IVF. Vande Wiele confiscated and froze the container holding the Del-Zios' eggs and sperm. As far as the Del-Zios were concerned, Vande Wiele had committed murder: they sued him and his employers for $1.5 million.

By coincidence, the Del-Zios' case against Vande Wiele was finally brought to trial in July 1978, the same month that Louise Brown was born. The birth of the world's first test tube baby put Shettles's early IVF attempt in a different light. After Brown's appearance, most people—including the two men and four women on the Del-Zio jury—seemed much more inclined to think of IVF as a medical miracle than as a threat to civilized society.

The trial lasted six weeks, each side making its case about the wisdom, safety and propriety of IVF. In the end, Vande Wiele was found to be at fault for "arbitrary and malicious" behavior, and he and his co-defendants were ordered to pay Doris Del-Zio $50,000.

IVF developed rapidly after the trial, and 200 more test tube babies—including Louise Brown's sister, Natalie—were born over the next five years. (Natalie is now a mother, having conceived naturally, and is the first IVF baby to have a child.) Seeing so many healthy-looking test tube babies worldwide changed Vande Wiele's opinion, a change that paralleled the transformation in feeling about IVF that was occurring in the public at large. When Columbia University opened the first IVF clinic in New York City in 1983, its co-director was Raymond Vande Wiele. *—R.M.H.*

COURTING JUSTICE: Doris Del-Zio and her attorney, Michael Dennis, outside U.S. district court in New York City on July 17, 1978, after a session of jury selection. Del-Zio and her husband, John, sued physician Raymond Vande Wiele for derailing their early attempt at in vitro fertilization.

AP PHOTO

before the Senate would explicitly protect research cloning while making reproductive cloning a federal offense.

IVF Risks Revealed

ONE RESULT OF the unregulated nature of IVF is that it took nearly 25 years to recognize that IVF children *are* at increased medical risk. For most of the 1980s and 1990s, IVF was thought to have no effect on birth outcomes, with the exception of problems associated with multiple births: one third of all IVF pregnancies resulted in twins or triplets, the unintended consequence of the widespread practice of implanting six or eight or even 10 embryos into the womb during each IVF cycle, in the hope that at least one of them would "take." (This brute-force method also leads to the occasional set of quadruplets.) When early studies raised concerns about the safety of IVF—showing a doubling of the miscarriage rate, a tripling of the rate of stillbirths and neonatal deaths, and a fivefold increase in ectopic pregnancies—many people attributed the problems not to IVF itself but to its association with multiple pregnancies.

By last year, however, IVF's medical dark side became undeniable. In March 2002 the *New England Journal of Medicine* published two studies that controlled for the increased rate of multiple births among IVF babies and still found problems. One study compared the birth weights of more than 42,000 babies conceived through assisted reproductive technology, including IVF, in the U.S. in 1996 and 1997 with the weights of more than three million babies conceived naturally. Excluding both premature births and multiple births, the test tube babies were still two and a half times as likely to have low birth weights, defined as less than 2,500 grams, or about five and a half pounds. The other study looked at more than 5,000 babies born in Australia between 1993 and 1997, including 22 percent born as a result of IVF. It found that IVF babies were twice as likely as naturally conceived infants to have multiple major birth defects, in particular chromosomal and musculoskeletal abnormalities. The Australian researchers speculate that these problems may be a consequence

THE DAY AFTER her 20th birthday, Louise Brown poses at home with her parents.

ADRIAN ARBIB *Corbis*

of the drugs used to induce ovulation or to maintain pregnancy in its early stages. In addition, factors contributing to infertility may increase the risk of birth defects. The technique of IVF itself also might be to blame. A flawed sperm injected into an egg, as it is in one IVF variation, may have been unable to penetrate the egg on its own and is thus given a chance it would otherwise not have to produce a baby with a developmental abnormality.

Clearly, these risks could remain hidden during more than two decades of experience with IVF only because no system was ever put in place to track results. "If the government had supported IVF, the field would have made much more rapid progress," says Duane Alexander, director of the National Institute of Child Health and Human Development. "But as it is, the institute has never funded human IVF research of any form"—a record that Alexander calls both incredible and embarrassing.

Although the medical downsides of IVF are finally coming to light, many of the more alarmist predictions about where IVF would lead never came to pass. For example, one scenario was that it would bring us "wombs for hire," an oppressed underclass of women paid to bear the children of the infertile rich. But surrogate motherhood turned out to be expensive and emotionally complex for all parties, and it never became widespread.

Human cloning, too, might turn out to be less frightening than we currently imagine. Market forces might make reproductive cloning impractical, and scientific advancement might make it unnecessary. For example, people unable to produce eggs or sperm might ponder cloning to produce offspring. But the technology developed for cloning could make it possible to create artificial eggs or sperm containing the woman's or man's own DNA, which could then be combined with the sperm or egg of a partner. In the future, "cloning" might refer only to what is now being called therapeutic cloning, and it might eventually be truly therapeutic: a laboratory technique for making cells for the regeneration of damaged organs, for example. And some observers believe that the most common use of cloning technology will ultimately not involve human cells at all: the creature most likely to be cloned may wind up being a favorite family dog or cat.

The history of IVF reveals the pitfalls facing cloning if decision making is simply avoided. But despite similarities in societal reactions to IVF and cloning, the two technologies are philosophically quite different. The goal of IVF is to enable sexual reproduction in order to produce a genetically unique human being. Only the site of conception changes, after which events proceed much the way they normally do. Cloning disregards sexual reproduction, its goal being to mimic not the process but the already existing living entity. Perhaps the biggest difference between IVF and cloning, however, is the focus of our anxieties. In the 1970s the greatest fear related to in vitro fertilization was that it would fail, leading to sorrow, disappointment and possibly the birth of grotesquely abnormal babies. Today the greatest fear about human cloning is that it may succeed. SA

MORE TO EXPLORE

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