The isolation of human embryonic stem cells could, in theory, allow scientists to cultivate any of the body's tissues in the lab, but realizing this potential won't be easy

Harnessing the Power of Stem Cells

When developmental biologist Gary Anderson walks into his lab at the University of California, Davis, in the morning, he's sometimes greeted by an amazing sight: a plate of cardiac muscle cells pulsing in rhythm. To Anderson, though, the sight is frustrating. He'd rather see no change at all: the same pig embryonic stem (ES) cells he left the night before, staying immature indefinitely. For reasons Anderson does not yet understand, however, they spontaneously differentiate into any of a variety of cell types—sometimes a disorganized blob of nervous tissue or cartilage, sometimes the heart cells. "We're constantly battling their desire to differentiate," Anderson says.

STEM CELLS

In this special Focus on stem cells, one News article deals with research aimed at controlling their development and another profiles a stem cell proponent, while a Perspective on p. 1468 outlines the medical potential of stem cells.

▶ CELL FATE PROFILE

That powerful drive to become something else is a signature characteristic of stem cells, the versatile cells that are one of the hottest and most controversial topics in developmental biology. The current excitement was touched off 5 months ago, when two groups reported that they had isolated the first lines of human ES cells—cells that can not only differentiate into all types of tissue, but can, under carefully controlled conditions, be maintained continuously as undifferentiated cells in lab cultures (*Science*, 6 November 1998, pp. 1014 and 1145). The work has sparked some controversy because one group extracted the cells from donated human embryos from a fertility clinic, while the other used tissue from aborted fetuses. But it could ultimately open the way to growing replacements for many types of tissue or even organs damaged by disease (see Perspective on page 1468).

That's what has made human ES cells such a hot commodity. But they are not the only kinds of stem cells that researchers have been cultivating. Over the past few years, scientists have also isolated stem cells from various tissues in animals and humans, including bone marrow and even brain, that are not "totipotent"—meaning that they can't form all tissue types, as ES cells do—but can produce a narrower range of cells, such as the various blood, muscle, or nerve cells. What's more, recent findings suggest that these partially differentiated stem cells may be more versatile than thought.

Whichever kind of stem cell they work with, researchers hoping to channel them into becoming certain kinds of tissue—say, dopamine-producing neurons for implantation into patients with Parkinson's disease, heart muscle cells to repair damaged hearts, or insulin-producing cells for diabetes patients—will have to solve a great many problems first. Put simply, they will need to understand and control the process of cell specialization that, for example, turns stem cells into beating heart cells in Anderson's lab.

HUMAN STEM CELLS ISOLATED

Stem cell type	Source	Daughter tissues
embryonic (ES)	embryo or fetal tissue	all types
hematopoietic	adult bone marrow	blood cells; brain?
neuronal	fetal brain	neurons; glia; blood?
mesenchymal	adult bone marrow	muscle; bone; cartilage; tendon

"Right now, we can get spontaneous differentiation," says Martin Pera, a developmental biologist at Monash University, in Melbourne, Australia, but "we don't understand how to control that process" to produce cells of a specific lineage.

To accomplish their goal of growing replacement tissues in the lab, researchers will have to identify the signals that tell a stem cell to become one tissue or another—one of the central mysteries in developmental biology. "We generally know the vocabulary" of growth factors and other molecules that help determine cell fates, says stem cell researcher Roger Pedersen of the University of California, San Francisco. "At least we know what to listen for." But development also requires direct communication between cells and their neighbors, and Pedersen adds, "Eavesdropping on the particular conversation going on between the small group of founder cells in the embryo and their environment is not easy." Once the conversation is known, researchers will have to take the next step: determining whether they can reproduce those signals for cells growing in lab cultures.

Blood and brains and bone

The challenges are large, but already researchers have some promising clues. Much of the success so far has come in directing the differentiation of the tissue-specific cells. For example, a decade after scientists first isolated the stem cells that form blood cells from mouse bone marrow, researchers have learned how to identify and select such hematopoietic stem cells from human bone marrow, support them as they develop into blood precursor cells, and then use an array of growth factors and other regulatory proteins to guide their development into red cells and other mature blood cells.

Scientists also have promising clues about how to guide the development of neuronal stem cells. Developmental neurobiologist David Anderson of the California Institute of Technology (Caltech) in Pasadena and his colleagues, working on cells derived from fetal rats, have shown that several proteins, including neuregulin and bone morphogenic protein 2 (BMP2), can nudge neural stem cells toward becoming neurons, the neuronal support cells known as glia, or even smooth muscle. The researchers don't yet know exactly how the molecules are working, however.

And it takes more than just the right molecules to get stem cells to differentiate. For example, researchers have found that even with the best neuronal growth factors known, they could at most get only about half of the neural stem cells in their cultures to become neurons. Developmental neurobiologists think that may be because a developing brain cell relies on a specific combination of signals from its neighbors, reflect-

ing its three-dimensional position in relation to other developing brain cells, to determine the specific type of cell it becomes.

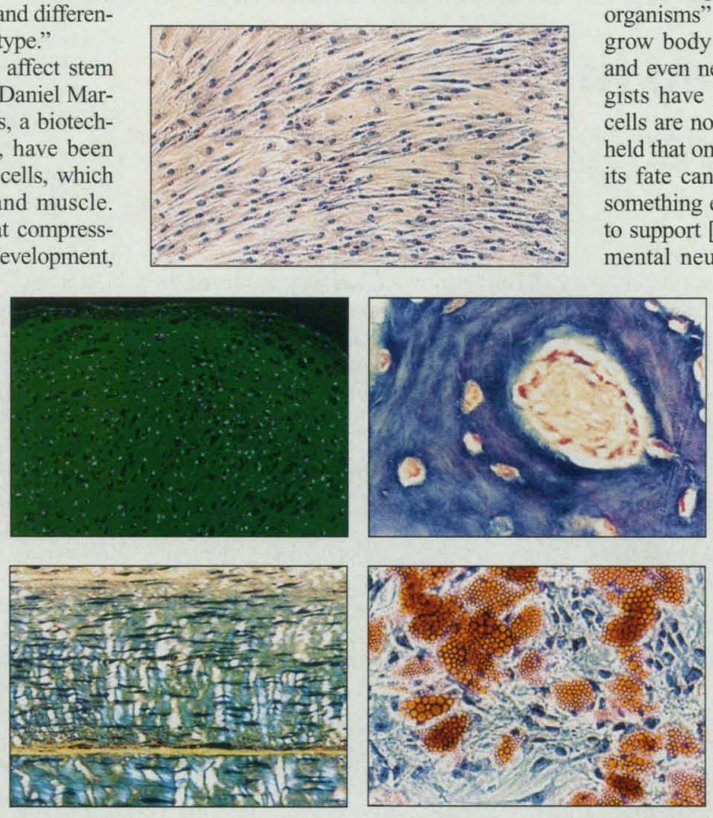
"Essentially, to induce that differentiation [into neurons], you need the other cell types present," says developmental geneticist Robin Lovell-Badge of the National Institute for Medical Research in London, who is among those working on the problem. "It's never going to be a simple story." Caltech's Anderson agrees: "We are far from being able to take a cell from the brain and differentiate it, at will, into a desired cell type."

Even mechanical forces can affect stem cell development. Cell biologist Daniel Marshak and his colleagues at Osiris, a biotechnology company in Baltimore, have been working on mesenchymal stem cells, which produce bone, cartilage, fat, and muscle. They and others have found that compressing the cells encourages bone development, while a tensile force—essentially stretching the matrix surrounding the cells—encourages development of tendons. Cartilage, on the other hand, will only form if the cells are clumped together or grown on a three-dimensional support, says Marshak.

Although scientists are not as adept at controlling the more primitive ES cells, they have made some progress there, too. Because human ES cells have been available only for a few months, most of this work has been done on mouse ES cells and cell lines from teratocarcinomas. These testicular or ovarian tumors grow from undifferentiated cells that have properties similar to those of ES cells. The tumors are masses of varied tissues, sometimes sprouting hair and even teeth, and although the cells are good for study, they aren't as promising for human therapies as ES cells are because of their tumor-forming potential.

So far, researchers have found that they can induce ES and teratocarcinoma cells to produce both neuronal and mesenchymal cells. In fact, some hints from developmental biologists suggest that neuronal cells may be a "default" fate for undifferentiated cells—meaning that's what they form when left to their own devices. Perhaps because of that, it is relatively easy to turn ES cells into neurons. Adding retinoic acid—which helps drive nervous-system development in the embryo—at the proper time "will make 90% of the cells go down the neural lineage path," says David Gottlieb of Washington University in St. Louis. Those cells, in turn, differentiate spontaneously into neurons and glia.

Producing mesenchymal cells from ES or teratocarcinoma cells requires a different signal: BMP4. With the right factors, Gottlieb says, "you can tilt their fate in the desired direction." No one is yet sure, however, which factors to use to encourage the formation of the third major type of tissue, endoderm, which gives rise to the gut, lungs, and inner organs. But developmental biologist Douglas Melton at Harvard University has been able to select a few mouse ES cells that differentiate spontaneously into endoderm cells.



Tissues in a dish. Mesenchymal stem cells *(top)* can become *(clockwise from upper left)* cartilage, bone, fat, and tendon cells.

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He can also persuade those endodermal cells to become pancreatic cell precursors—just a few steps away from the insulin-producing cells needed by diabetes patients—by exposing them to cells from the "pancreatic bud" dissected from another mouse. Those cells "send out signals saying, 'Come join us and become pancreas,' " Melton says. Of course, to make human pancreatic cells, scientists would have to reproduce those signals without the help of already-developing tissue, since work with fetal tissue raises serious ethical, moral, and perhaps legal issues, Melton notes.

Unexpected versatility

But ES cells may not be the only hope for cultivating a wide range of tissues. There are signs that the more differentiated kinds of stem cells might have greater potential for tissue replacement than researchers had assumed. In a major surprise that seems to have overturned some long-standing beliefs about developmental biology, scientists reported earlier this year that neural stem cells from the mouse brain, when transplanted into mice whose bone marrow has been largely destroyed, develop into blood cells (*Science*, 22 January, pp. 471 and 534).

Although amphibians and other "lower organisms" can reprogram their cells to regrow body parts—new tails, for example, and even new limbs—developmental biologists have long assumed that mammalian cells are not so versatile. The standard view held that once a more specialized cell forms, its fate can't be altered so that it becomes something else. But "there is very little data to support [that assumption]," says developmental neurobiologist Ron McKay of the National Institute of Neurological Disorders and Stroke. The new observations suggest "that cells can make unexpected jumps between fates," he says. "It's going to be interesting to see where those jumps can occur."

Neuronal stem cells may not be the only ones able to switch fates. Last year, for example, developmental biologist Darwin Prockop of the Allegheny University of the Health Sciences in Philadelphia and his colleagues reported in the *Proceedings of the National Academy of Sciences* that when bone marrow stromal cells that would normally form muscle and other mesenchymal tissues are injected into the brains of mice, the cells become glia. And Juan Sanchez-Ramos of the University of South Florida in Tampa and his colleagues have preliminary evidence, presented at a meeting last year, that exposure to neural growth factors in culture can also send marrow cells down the road to becoming neurons.

Many stem cell researchers are greeting the neuronal stem cell–to–blood cell results with caution, however. "It's a preliminary analysis," says developmental hematologist Leonard Zon of Children's Hospital in Boston. The blood cells may have grown not from neuronal stem cells, he says, but from a more primitive stem cell also in the sample.

But if the results are confirmed, the stem cell versatility could open the way to cell therapies that don't rely on embryonic stem cells, with their ethical and legal entanglements. A sample of a patient's own bone-marrow cells, drawn through a needle insert-

ed into the hip bone, could be manipulated in culture and perhaps used to replace neurons in the brain or damaged heart muscle.

Such applications, however, face the same hurdles as other potential stem cell treatments. More immediately exciting, say many scientists in this area, is the chance to study what it is about a cell that allows it to remain malleable and able to change its fate in response to environmental cues.

Indeed, the efforts to harness stem cells' potential for biomedical applications is a boon for cell and developmental biologists. With cultured stem cells, cellular changes "that have only occurred in the complex context of the early embryo are now happening before your eyes in a dish," says Washington University's Gottlieb. And those changes seem to be very similar to the ones that happen in the developing embryo, producing normal-looking neurons that make synapses and heart cells that set up rhythms. "What we're looking forward to is a much greater level of understanding and control," he says. "It's a field in which chapter one has been written. I'm looking forward to chapter two."

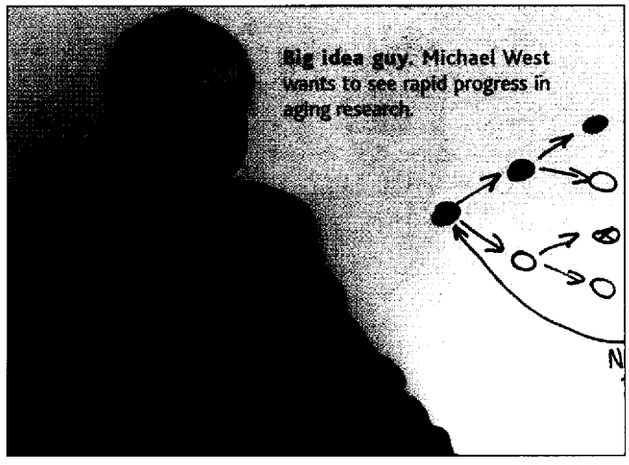
–GRETCHEN VOGEL

STEM CELLS
PROFILE

A Man in a Hurry

While pushing research on stem cells and other areas that might help an aging population, Michael West has also sparked controversy

For Michael West, the man whose support of stem cell research led to three dramatic announcements last fall, aging is a driving force. But it's not so much his own aging that worries him. Instead, it's the fact that the population as a whole is growing older, placing an increasing demand on the nation's health care system. "Addressing the medical problems associated with aging has got to become our number-one priority for research in this country," says West, who is president and chief executive officer of Advanced Cell Technology (ACT) in Worcester, Massachusetts.



Big idea guy. Michael West wants to see rapid progress in aging research.

And because aging is relentless, West himself has been relentless in funding and promoting cell technologies that could rejuvenate diseased tissues. In so doing, he has earned praise for his vision and energy as well as criticism for his bulldog approach. Although he's now heading up ACT, West is the founder of Geron Corp. of Menlo Park, California, a biotechnology firm that funded the two groups that last November reported isolating human embryonic stem (ES) cells (*Science*, 6 November 1998, pp. 1014 and 1145). These cells might have wide application in the clinic because they can be maintained in lab culture and, theoretically at least, can differentiate into all types of tissues (see p. 1432).

"Mike is a big idea guy," says Jerry Shay, a cell biologist who worked with West at the University of Texas Southwestern Medical Center in Dallas, "but he is also a guy willing to do what needs to be done to make sure that important ideas actually get translated into action." To West, that sometimes means courting controversy.

The ES cell work had already stirred debate because one group extracted the cells from aborted fetal tissue and the other from embryos discarded by a fertility clinic. Barely a week later, West added more fuel to the fire. He announced that ACT cofounder James Robl of the University of Massachusetts, and his then graduate student José Cibelli, had produced what appeared to be ES cells by first fusing human nuclei with cow oocytes whose own nuclei had been removed and then allowing these hybrid cells to divide. (See *Science*, 20 November 1998, p. 1390.) West announced this, he says, because he was leery of the legality of the work, and wanted to gauge the reaction of both the public and the U.S. government before he committed company funds to continue this research.

And react they did. President Clinton asked the National Bioethics Advisory Committee (NBAC) to review the medical and ethical aspects of the work. And much of the scientific community slammed him for publicly discussing data that had not yet been published in a peer-reviewed journal—stirring public debate about results that were not even documented. "I argued at length with Mike about going public with this," says John Gearhart at Johns Hopkins University School of Medicine, one of the two researchers whose work on stem cells West supported while at Geron. "First, you don't publish scientific work in *The New York Times*, and second, I think this approach of doing interspecies research is inappropriate."

But West shrugs off the criticism. "NBAC's immediate review found that there were no totally new ethical issues raised by this research as long as it is not intended to produce an embryo with the potential to develop into a child," which West says the hybrid cells could not do in any case. "As a result, we're now free to pursue this avenue of research aggressively, and we do intend to publish our results in the scientific literature. "

West wasn't always driven to cure the ills of aging. In fact, he got a slow start on a scientific career. After graduating from Rensselaer Polytechnic Institute in 1975, West went to work in the family business selling trucks, which he eventually parlayed into a truck-leasing business. He soon became interested in gerontology, especially the question of why the ordinary somatic cells of the body have a finite lifetime. When he sold the truck business, he was free to turn to science. "I made a fair amount of money on that transaction, which has given me the freedom to do what I've wanted to do in science without having to worry about making a living," says West.

A false start in graduate school at the University of Arkansas for Medical Sciences—he showed that one of his Ph.D. adviser's papers was incorrect—further delayed his research career. But he obtained his Ph.D. at Baylor College of Medicine in 1988, then decided to go to medical school there. He never finished, however, because his interests took a new turn.

At Baylor, he met Jerry Shay and longtime colleague Woodring Wright, who were among those who had linked shortening of the telomeres, specialized DNA sequences capping the ends of the chromosome, to cellular aging. The idea is that when the telo-

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