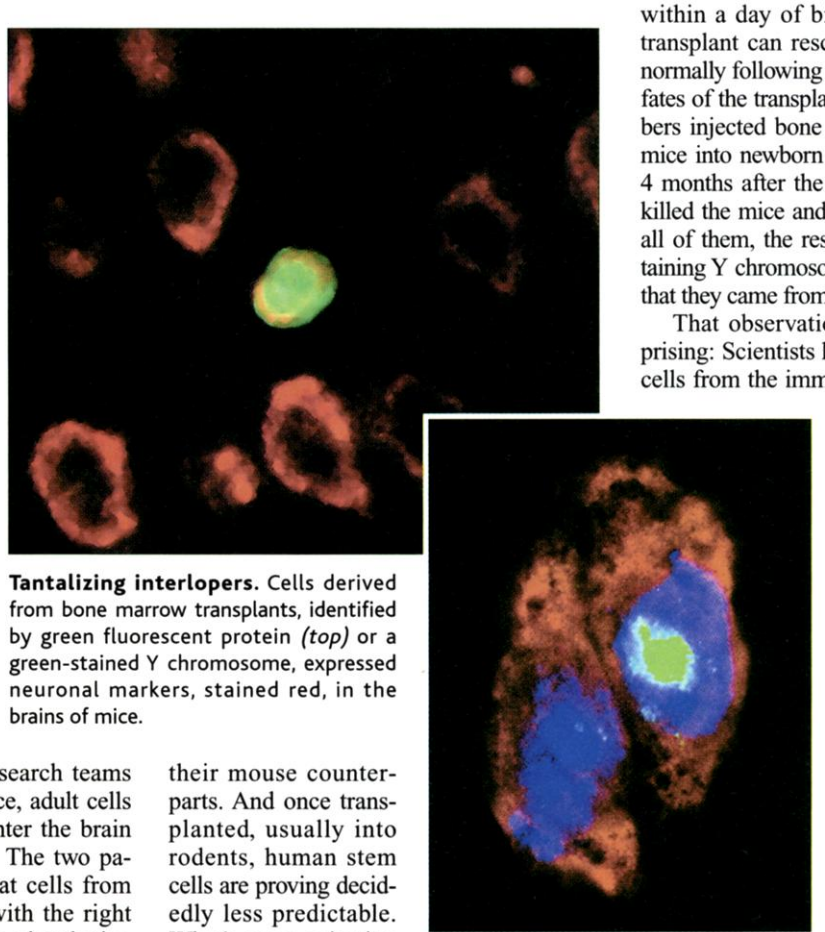
Bone marrow cells that become neuronlike cells in the brain fascinate scientists, but ample uncertainties must be resolved before such results can be translated into therapeutics

Stem Cells: New Excitement, Persistent Questions

If only bodies were as easy to fix as automobiles, diseases like diabetes or heart disease would be vanquished. Worn-out, defective cells would be readily replaced, new organs inserted, and impossible illnesses cured. Sometimes, such a world seems just around the corner—if you don't read the caveats too closely. Almost each week brings another report of the uncanny abilities of versatile stem cells, when transplanted into mice or rats, to form new blood vessels, strengthen weak bones, and even seek out and begin to repair damaged spinal cords and brains (*Science*, 24 November, p. 1479).

Much of the excitement has focused on the ability of these partially developed cells present in early embryos, fetal tissue, and several adult tissues to change course and become different types of cells—a proto–brain cell morphing into a muscle cell, say, or a bone marrow cell into a liver cell. For many years researchers assumed that a cell's fate was sealed, irrevocably, early in development. But increasingly, experiments are undermining that idea. In the latest example, two independent research teams report in this issue that, in mice, adult cells from the bone marrow can enter the brain and become neuronlike cells. The two papers strengthen the notion that cells from adult tissue, when prodded with the right signals, can change trajectories, abandoning their original identity and assuming a new one. If a similar phenomenon occurs in human brains—still a big if—it could mean that easily accessible cells from bone marrow might someday be used to treat a wide range of neurological diseases—without raising the ethical concerns that accompany the use of embryonic cells.

But there's a catch. Can the dramatic findings that so far have grown out of work with stem cells taken from mice be repeated in humans? Research on human cells lags behind, in part because of ethical debates restricting the use of cells derived from human embryos and fetuses (see sidebar), but also because of certain characteristics of human cells themselves. Human cells grow more slowly and divide less often in culture than their mouse counterparts. And once transplanted, usually into rodents, human stem cells are proving decidedly less predictable. What's more, scientists are at a loss to explain the surprising behavior of both human and mouse stem cells. The molecules that control the unusual fate-switching and tissue-rescuing cells remain elusive, making it difficult to test the observations with human cells, especially, in culture. Any human treatments, suffice it to say, are years away.



Tantalizing interlopers. Cells derived from bone marrow transplants, identified by green fluorescent protein *(top)* or a green-stained Y chromosome, expressed neuronal markers, stained red, in the brains of mice.

The latest papers highlight the personality-switching abilities of mouse stem cells while also reflecting some of the uncertainty typical of the field. Éva Mezey of the National Institute of Neurological Disorders and Stroke (NINDS) and her colleagues describe on page 1779 how they transferred bone marrow cells from normal adult mice into a strain of mice that cannot produce immune system cells. Usually, mice without immune systems die within a day of birth, but a bone marrow transplant can rescue them, and they grow normally following the transplant. To trace the fates of the transplanted cells, the team members injected bone marrow from adult male mice into newborn female recipients. One to 4 months after the transplants, the scientists killed the mice and examined their brains. In all of them, the researchers found cells containing Y chromosomes—unmistakable proof that they came from the male donors.

That observation in itself was not surprising: Scientists have known for years that cells from the immune system can enter the brain, and recent reports have shown that cells present in bone marrow could become astrocytes and glia, the brain's supporting cells. The unexpected result was that a small percentage of the male-derived cells expressed protein markers typical of neurons, the brain's key signaling cells, suggesting that the bone marrow cells had, upon reaching the brain, become neurons. Until a few years ago, scientists did not think mammals produced any new neurons at all after childhood—much less that foreign bone marrow cells could be coaxed into such a feat.

In independent work, cell biologist Helen Blau, graduate student Tim Brazelton, and their colleagues at Stanford University also found evidence for the versatility of adult bone marrow cells. As reported on page 1775, the team members injected bone marrow cells from adult mice into otherwise nor-

CREDITS: (TOP TO BOTTOM) T. BRAZELTON; É. MEZEY

Stem Cell Scorecard

As researchers continue to explore the potential uses of stem cells obtained from a variety of sources (see main text), governments around the world are grappling with whether to allow research on stem cells derived from human embryos. Governments are cautious yet increasingly open to the new research, which may eventually yield treatments for a variety of diseases from Parkinson's to diabetes.

Japan: The Council for Science and Technology, the country's highest scientific advisory group, was scheduled to begin discussions of final guidelines governing the use of stem cells this week. Those guidelines are expected to closely resemble a draft document released last spring (*Science*, 11 February, p. 949). Until those guidelines are in place, scientists in Japan are not allowed to derive or work with human embryonic stem (ES) cells, says Shin-Ichi Nishikawa of the University of Kyoto. One use of the technology is likely to be banned: A law prohibiting human reproductive cloning has passed the lower house of parliament and is expected to pass the upper house, says Koichi Morimoto, science counselor at the Japanese embassy in Washington, D.C.

Germany: Although German law forbids research that harms an embryo, it does not prohibit import of already-derived ES cells, according to Oliver Brüstle of the University of Bonn. Brüstle has applied for a grant to do just that and is waiting to hear back from Germany's funding agency, the DFG. Legislation is unlikely to change in the near future, he says.

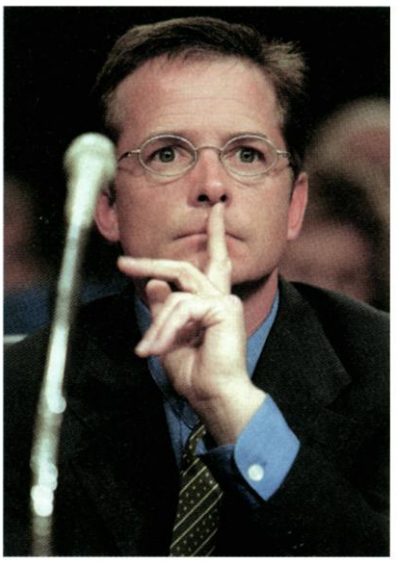
Sweden: The government is reviewing its guidelines for stem cell and cloning research, even as plans are under way at Stockholm's Huddinge Hospital to launch a project to derive new stem cell lines from embryos that will be available for basic research, says neuroscientist Anders Bjorklund of Lund University.

United Kingdom: The U.K. may be the most permissive. In a report supported by Prime Minister Tony Blair, an advisory committee recommended in May that researchers be allowed to conduct nuclear transfer experiments with human cells (*Science*, 25 August, p. 1269). Some scientists would like to learn how to transfer the nucleus from a patient's cell into an enucleated egg in order to derive perfectly matched stem cells for treating the patient. Parliament is expected to debate revisions to the law governing research on embryos in the next few months.

Australia: In Australia, policies are in flux. State law varies across the country. Victoria, for example, prohibits the derivation of ES cells, but other states have no regulations. The National Health and Medical Research Council, the country's biomedical funding agency, has issued guidelines on research in human-assisted reproduction technology; these state that human ES cells may not be developed with the aim of cloning an individual. A National Parliamentary Committee is currently considering the status of therapeutic cloning, stem cell research, and related matters. They are expected to report before the end of the year.

European Union: An E.U. ethics advisory board recommended in November that the E.U. fund research using all types of stem cells, especially those derived from adult tissue. Because research on ES cells is still preliminary, the advisory board discouraged work that would create new embryos for research. Plenty of "excess" embryos already exist in fertility clinics, destined to be discarded, the report says.

United States: Although the National Institutes of Health (NIH) issued guidelines for funding work with human pluripotent stem cells in August, federally funded researchers will not be able to begin such work until next spring at the earliest. The guidelines allow NIH-funded scientists to use embryonic or fetal stem cells only after careful ethical review of the methods used to derive those cells. Because no scientist has yet submitted all the documentation needed for the review, says NIH associate director for policy Lana Skirboll, the Human Pluripotent Stem Cell Review Group will not meet this month as originally scheduled. Committee members should be announced before year's end, Skirboll says, and the next scheduled meeting is in April 2001.



Hopeful. Actor Michael J. Fox urged a congressional committee to approve research on embryonic stem cells that may someday cure Parkinson's disease.

That will be well into the new presidential Administration, and if Governor George W. Bush prevails, the climate at NIH may change. Bush has stated that he is opposed to the NIH funding work with ES cells, and a Bush-appointed director could prohibit any ES cell work.

Such an outcome could prompt stem cell supporters in Congress to act. Senator Arlen Specter (R–PA), for instance, last year introduced a bill that would have authorized the NIH to fund work on the derivation and use of human ES cells. But despite star-studded hearings in which actors Mary Tyler Moore, Michael J. Fox, and Christopher Reeve testified in favor of the research, the bill died in the Senate (*Science*, 13 October, p. 261). Specter has said he will reintroduce the bill in the new Congress. **–G.V.**

mal mice that had received a lethal dose of radiation to kill their bone marrow cells. The researchers used bone marrow from mice genetically engineered to express green fluorescent protein in their cells so they could track the injected cells. Several months after the transplant, the researchers found glowing green cells throughout the brains of recipient mice. To determine what type of brain cells the bone marrow had become, the team members stained brain sections to detect neuronal-type markers. To their surprise, they, too, found transplant-derived cells expressing multiple neuronal proteins.

Despite both teams' independent results, other scientists caution that protein markers can be misleading. Mature, functional neurons can be notoriously difficult to identify using cell markers, and both teams failed to detect more than a few cells with the characteristic shape of a mature neuron, with long extensions reaching out to other cells. The transplanted cells are "expressing certain features of neurons, but there's a lot we don't know," says developmental neuroscientist Ron McKay of NINDS.

And if the cells truly are neurons, the scientists still need to decipher exactly which bone marrow cells enter the brain and what molecular signals draw them there. Neuroscientist Anders Bjorklund of Lund University in Sweden suspects that the age and condition of the recipient mouse might influence the recruitment of bone marrow cells to the brain. Mezey and her colleagues worked with newborn mice, and it might be easier for stem cells to infiltrate those still-developing brains. In Brazelton and Blau's work, the adult recipient mice received a

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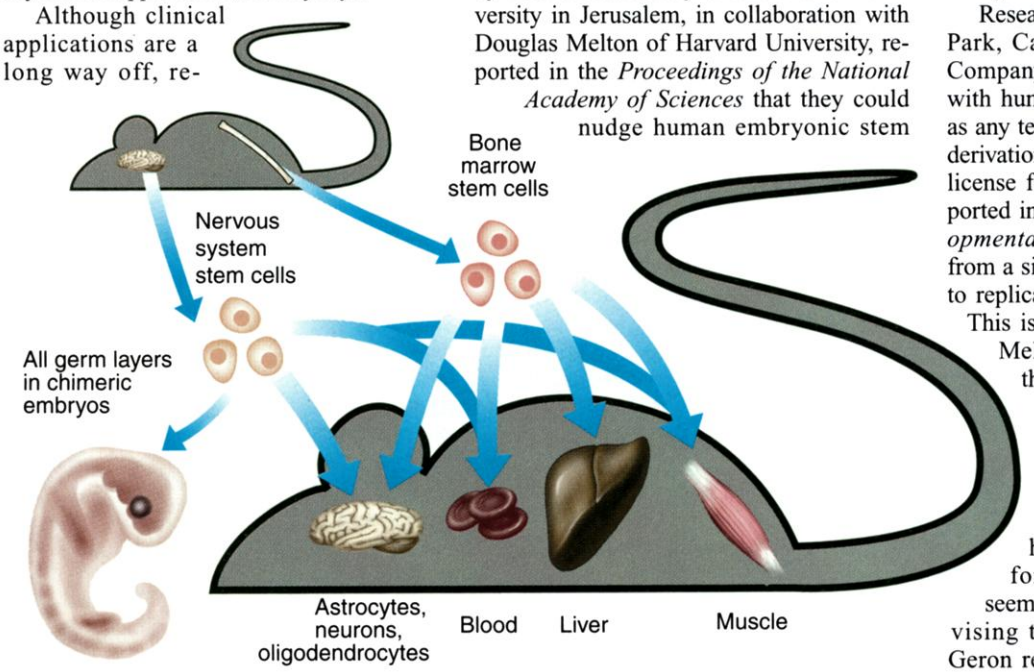
high dose of radiation that killed not only bone marrow but also any dividing cells in the brain. Perhaps such an assault prompted the migration of cells, Bjorklund speculates.

Blau's team is now working to characterize the molecules that control the recruitment process. "We need to find out what factors we can deliver to make cells divide and home in and take up residence in the right place," she says. Indeed, a detailed understanding of such factors would probably have to precede any clinical applications, McKay says.

Although clinical applications are a long way off, recent work supports the idea that human bone marrow might also have multiple talents, although exploiting them may still be a challenge. Researchers led by Darwin Prockop of Tulane University in New Orleans and Ira Black of Robert Wood Johnson Medical School in Piscataway, New Jersey, reported in the August *Journal of Neuroscience Research* that human marrow stromal cells, a subset of bone marrow, began to resemble neuronal-type cells in culture. And this summer, Malcolm Alison of the Imperial College School of Medicine in London and his colleagues reported in *Nature* that at least a few human bone marrow cells became liver cells in patients who had received bone marrow transplants. In the study, women who had received bone marrow transplants from male donors had liver cells that contained Y chromosomes—most likely derived from the transplanted bone marrow cells. That finding is consistent with previous reports of similar phenomena in mice (*Science*, 14 May 1999, p. 1168), suggesting that bone marrow cells might someday be useful in treating liver disease.

In contrast, the human embryonic stem cells and fetal germ cells that made headlines in November 1998 because they can, in theory, develop into any cell type have so far produced relatively modest results. Only a few papers and meeting reports have emerged from the handful of labs that work with human pluripotent cells, whose use has been restricted by legal and commercial hurdles. Last month, a group led by Nissim Benvenisty of The Hebrew University in Jerusalem, in collaboration with Douglas Melton of Harvard University, reported in the *Proceedings of the National Academy of Sciences* that they could nudge human embryonic stem cells toward a number of different cell fates. But the results did not produce easy answers; some cells expressed markers from several kinds of lineages.



A web of possibility. In mice, stem cells from adult brain and bone marrow have shown encouraging potential for repairing a variety of tissues and organs and perhaps curing disease.

The work suggests that it will not be simple to produce the pure populations of certain cell types that would be required for safe and reliable cell therapies—much less the hoped-for replacement organs, says stem cell researcher Oliver Brüstle of the University of Bonn in Germany. Brüstle was one of the first to show that mouse embryonic stem cells could help treat an animal disease model, in which neurons lack their insulating coat of myelin. Even so, he is cautious about the near-term prospects in humans. Says Brüstle: "At present, it looks like it is really difficult to differentiate these [human] cells into more advanced cell types." Melton agrees. "It's unlikely anyone will ever find a single growth factor to make a dopaminergic neuron," as some might have hoped, but the work provides "a starting place," he says.

Simply keeping human embryonic stem cells alive can be a challenge, says Peter Andrews of the University of Sheffield in England. For more than a year, he and his colleagues have been experimenting with embryonic stem cell lines that James Thomson derived at the University of Wisconsin, Madison. "They're tricky," Andrews says. It took several false starts—and a trip to Wisconsin—before the researchers learned how to keep the cells thriving, he says. Melton uses almost the same words: Human embryonic stem cells "are trickier than mouse," he says. "They're more tedious to grow."

Researchers from Geron Corp. in Menlo Park, California, are having some luck. Company researchers have been working with human embryonic stem cells as long as any team has, because Geron funded the derivation of the cells and has an exclusive license for their commercial use. They reported in the 15 November issue of *Developmental Biology* that cell lines derived from a single embryonic stem cell continue to replicate in culture for 250 generations. This is important, says Geron researcher Melissa Carpenter, because it means that a single human embryonic stem cell, which might be modified in the lab, could produce an essentially unlimited supply of cells for therapy. That was known for mouse embryonic stem cells but had not been shown in humans before. Even so, Geron researchers seem no closer than other groups to devising therapeutic uses for stem cells. Geron researchers reported last month at the annual meeting of the Society of Neuroscience that they had attempted to transplant human embryonic stem cells into rats. When they injected undifferentiated cells into the brain, they did not readily differentiate into brain cells, the researchers found. Instead, they stayed in a disorganized cluster, and brain cells near them began to die. Even partially differentiated cells, the team reported, tended to clump together; again, nearby brain cells died.

Still, Melton is optimistic. "How easily can we translate what we know in the mouse to the human? There's nothing we've found that makes me think it can't be done," he says.

The most important next step, say several stem cell researchers, is to identify the molecular processes that underlie the impressive feats of stem cells. Many of the purported breakthroughs are simply observations, Bjorklund says, which may eventually be explained by events unrelated to stem cell versatility. "That is going to be one challenge for those working in the field," he says. "One has to come up with a deeper understanding of the mechanisms involved to get anywhere." Blau agrees: "We have to understand the rules" to find out how to better play the cell-replacement game. **–GRETCHEN VOGEL**

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