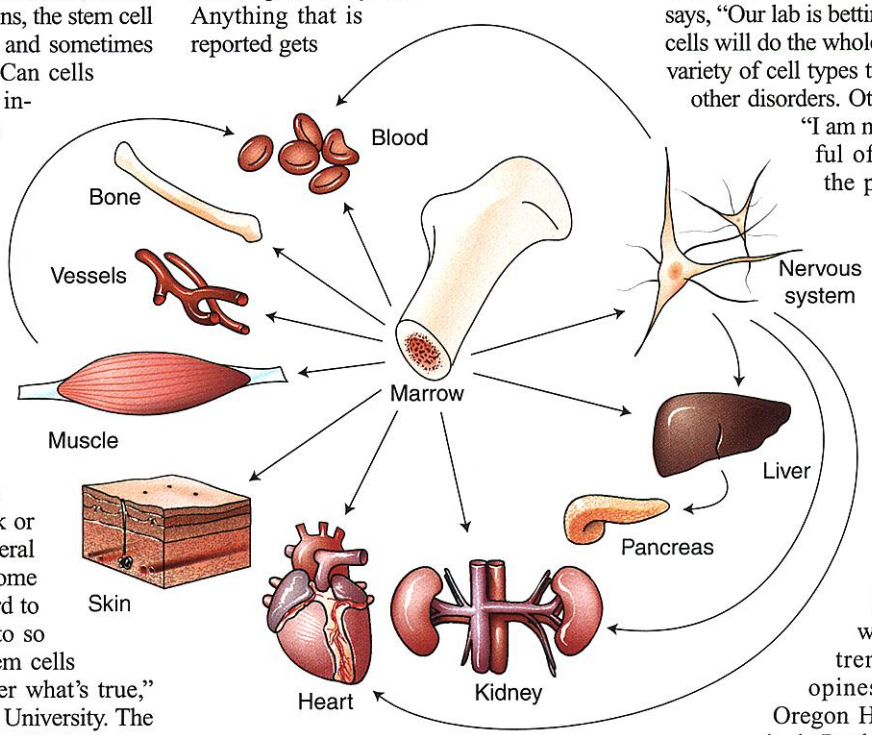
Long-standing biological dogma—that a cell, once committed, can't alter its fate—has been challenged by recent research. But now scientists are taking a more critical look

Plasticity: Time for A Reappraisal?

Although not quite the cold fusion of biology, it ignites similar passions. Stem cell "plasticity"—the ability of cell types from adult tissues to take on surprising new identities—can inspire debates as heated as any about that infamous physics claim. In hallways during conference coffee breaks, in strongly worded journal commentaries, and in behind-the-scenes conversations, the stem cell community is picking apart, and sometimes battling over, the evidence: Can cells from one type of tissue be induced to look and act like cells from a different tissue? Do these switches happen naturally? And could such transformations be used to treat deadly diseases?

A host of recent papers has suggested that stem cells from various adult tissues can, indeed, be reprogrammed—in defiance of age-old dogma that once a cell has become specialized, it can't backtrack or adopt a new identity. But several years after the first reports, some of the results are proving hard to replicate. "We have gone into so many traps recently with stem cells that I don't know any longer what's true," says Ole Isacson of Harvard University. The political stakes are enormous. If adult stem cells turned out to be so multitalented, they would offer a less controversial alternative to the use of embryonic stem (ES) cells. Ideally, a relatively easily accessible tissue such as skin or bone marrow could be cultivated into neurons, whose own stem cells are hard to reach, or blood, for which stem cells have proved hard to grow in culture.

Most scientists say no evidence yet suggests that adult stem cells can match the versatility of those derived from embryos, although work by marrow stem cell researcher Catherine Verfaillie of the University of Minnesota, Twin Cities, on a possible new type of cell might come close. And those cases in which plasticity seems to have occurred are so rare that the phenomenon might never be of practical use. Nonetheless, opponents of ES cell research have seized on any suggestion of plasticity in adult stem cells, often hyping it to make their case that ES cells are unnecessary (*Science*, 8 June 2001, p. 1820). Says Ihor Lemischka of Princeton University, "It's dangerous to talk about plasticity. ... Anything that is reported gets immediately translated into arguments pro and con in the White House."



Too good to be true? Studies in mice have yielded evidence, now being reassessed, that stem cells from a variety of tissues can produce progeny in different organs. Bone marrow, which has several types of stem cells, seems particularly versatile.

New paradigms?

Decades of experiments have shown that as animal cells divide during development, they take on new roles in an orchestrated way with little sign of haphazard fate-switching. That's why developmental biologists have been so surprised by recent claims of "transdifferentiation" or "plasticity," as the as-yet-unexplained observations of fate-switching are alternatively called.

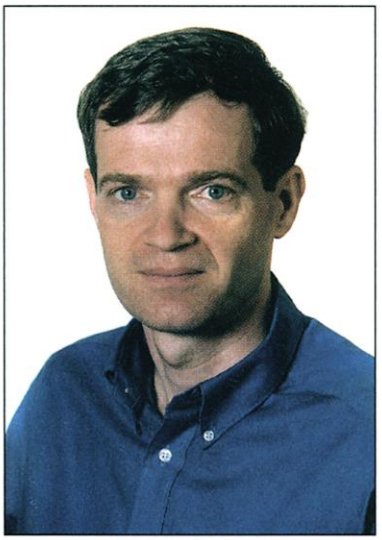
Some scientists are quite happy to question dogma. For instance, Darwin Prockop of Tulane University in New Orleans, Louisiana, who works with bone marrow–derived stem cells called mesenchymal cells, says, "Our lab is betting on the idea that these cells will do the whole job"—that is, supply a variety of cell types to treat neurological and other disorders. Others are more cautious: "I am not going to take a handful of papers" generated in the past few years and use them as a basis to dismiss decades of careful work, says Lemischka.

Plasticity was such a hot phenomenon in the late 1990s that journals, including *Science*, were snapping up and publishing partial—or what are now seen as questionable—results. "A lot of the big journals were willing to take extremely marginal stuff," opines Markus Grompe of Oregon Health & Science University in Portland.

As the political stakes have gone up, scientists have begun to pay closer attention to the multiple ways cells can fool them. For instance, cells in culture can mutate and develop markers characteristic of other lineages. Cells injected into foreign tissue can take up local DNA—and thus appear to have changed identity—without actually becoming transformed. An introduced macrophage can show the markings of a cell it's eaten. Or local residents might be mistaken for visitors. Margaret Goodell of Baylor College of Medicine in Houston, for example, discovered that what she thought were muscle stem cells turning into blood-forming cells were actually hematopoietic cells already residing in muscle.

ILLUSTRATION: C. SLAYDEN

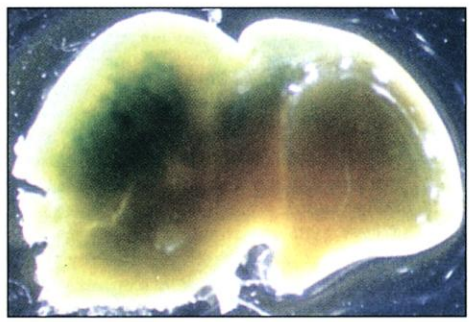
Hints that the picture might be a lot more complicated than some have assumed came last March, when research suggested that some apparent reprogramming of adult cells might instead be a case of cell fusion (*Science*, 15 March, p. 1989). Austin Smith of the University of Edinburgh, U.K., and Naohiro Terada of the University of Florida College of Medicine in Gainesville showed that when they cultivated adult bone marrow or brain cells with ES cells, the two cell types occasionally formed hybrids that looked like ES cells—but that had twice the usual numbers of chromosomes and were thus likely to be genetically unstable. The papers challenged only those plasticity observations that involve coculturing two types of cells. And the fusion danger might be limited to the highly volatile embryonic cells. Nonetheless, the findings were a wake-up call to several teams. "The fusion possibility never even entered our minds," Goodell admitted at a stem cell meeting held in March in Keystone, Colorado.



Wake-up call. Austin Smith of the Univeristy of Edinburgh led one of two teams that have raised the specter of cell fusion. Pictured here: chimeric mouse embryo with fused cells (blue).

The fusion scare has given further impetus to efforts to establish rigorous standards for demonstrating plasticity. Irving Weissman of Stanford University—one of the most outspoken skeptics in the field—with David Anderson of the California Institute of Technology in Pasadena and Fred Gage of the Salk Institute for Biological Studies in La Jolla, California, laid out in the April 2001 issue of *Nature Medicine* a set of requirements. First, the cells must be properly identified at the outset, because a single alien cell in ostensibly purified culture could produce misleading results. Putting out a few new proteins does not count, they said; instead, the cells must contribute to the functions of the host tissue: passing electrical signals in the nervous system, or filtering impurities from blood in the liver. This means that a single well-characterized donor cell has to be shown capable of creating a "robust" population and not just a scattering of cells in the new tissue. The trio also argued that the plasticity should be a natural phenomenon, which means cells must perform in the host tissue without having been altered in culture.

Others in the field agree that cells need to be better characterized and that they must demonstrate functionality. But beyond that, agreement breaks down on how many hoops these cells must jump through. The "gold standard" for demonstrating functionality was set by experiments done since the 1960s in which transplants of single blood stem cells recreated an entire blood system in lethally irradiated mice. But the blood system is one of the only tissues where such a dramatic demonstration of functionality is possible. Demonstrating that a particular neuron is functional in a brain, on the other hand, is difficult even for neuroscientists, notes Helen Blau of Stanford University, who did some of the earliest work in the 1980s suggesting that cells could switch lineages. "Yes, it's a good goal," she says. "But it's not an easy goal."



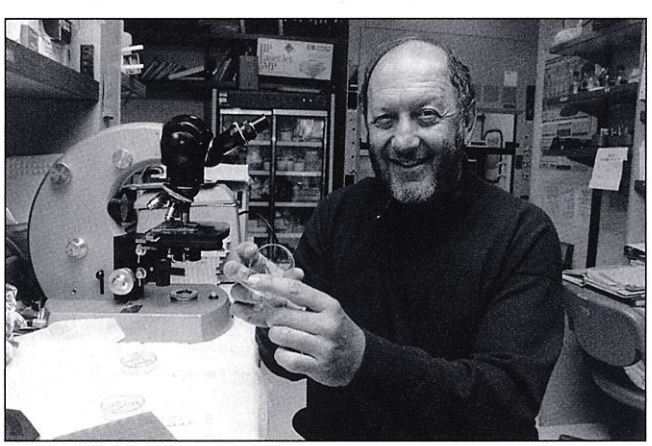
As for the "no-alteration-in-culture" requirement, Jonas Frisén of the Karolinska Institute in Sweden says that is relevant only if the goal is to study normal physiology. "If you're studying what is possible, then it's absolutely OK to culture." In fact, he adds, cells that are creatures of the test tube could prove useful in treating disease.

Do studies pass muster?

None of the studies so far purporting to demonstrate plasticity measures up fully against Weissman, Anderson, and Gage's rigorous criteria. For example, Diane Krause of Yale University and colleagues performed a complex two-stage study in which they injected dye-tagged male mouse blood stem cells into irradiated females. Then, to ensure that they were dealing with one particular type of cell, the researchers killed these mice and pulled out tagged cells that had homed in to the bone marrow. They then implanted a single cell into each of a second group of female mice. The researchers reported in the 4 May 2001 issue of *Cell* that some of the progeny of these cells became incorporated into various tissues including lung, skin, intestine, and liver, as well as bone and blood. Thus, Krause met two requirements: She used cells that had not been altered in culture, and she showed that a single blood stem cell can give rise to multiple cell types.

But Krause is now checking for evidence of cell fusion. And Eric Lagasse of StemCells Inc. in Palo Alto, California, is skeptical, noting that the cells plucked from the first transplant recipient were "not very well characterized." Verfaillie adds that the studies "still don't really show significant contribution to any organ. They're just small groups of cells without any function." Finally, the experiment hasn't yet been replicated. Weissman reported at a June meeting in Stockholm at the Karolinska Institute that when he and his team tried to repeat the result with carefully screened blood stem cells, they found only the expected bone and blood derivatives, six liver cells (too few to be significant, he says), and one brain cell. And the brain cell, a large type called a Purkinje cell, had twice the normal complement of DNA—meaning that it could have been a local cell that fused with one of the tagged cells. "There is no evidence that purified [blood] stem cells can contribute to any other tissue," he says—although Krause counters that her team's precursor cell might be different from the one Weissman tested.

Failure to replicate has put one of the earliest, most provocative studies (*Science*, 22 January 1999, p. 534) on shaky ground as well. Christopher Bjornson of the University of Washington, Seattle, Angelo Vescovi of the National Neurological Institute in Mi-



Voice of caution. Stanford's Irving Weissman is probably the most outspoken critic of plasticity claims.

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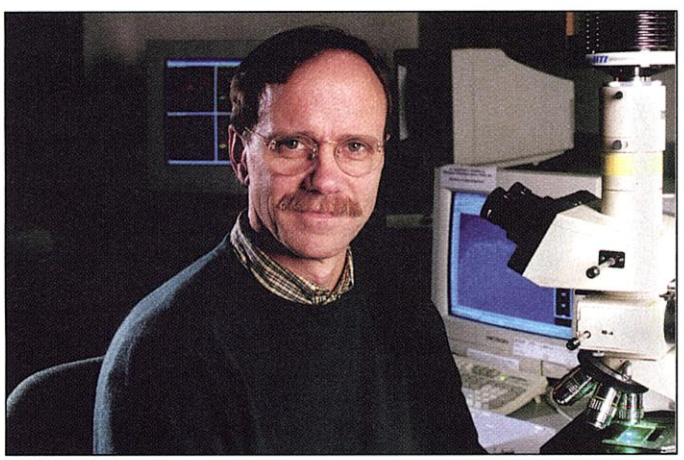
lan, Italy, and their colleagues reported that cultured mouse neural stem cells appeared to produce a variety of blood cell types after being planted in irradiated mice. When Derek van der Kooy of the University of Toronto repeated the protocol, using a population of neural stem cells derived from a single progenitor cell, he failed to generate any blood cells, he reported in the February issue of *Nature Medicine*.

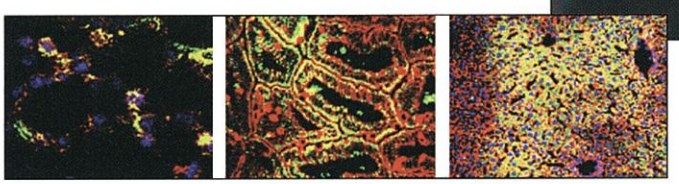
Two years ago, a study by Karolinska's Diana Clarke, Frisén, and their colleagues also seemed to support the idea that brain cells have broad potential. But it, too, is meeting skepticism. The Karolinska team purified neural stem cells in culture, allowed them to grow into cell clumps called neurospheres, and inserted them into the blastocyst of a developing mouse embryo. These cells, marked with a blue dye, contributed to development in all three germ layers in the animal, the team reported in the 2 June 2000 issue of *Science* (p. 1559). Although Frisén concedes that some of the apparently reprogrammed cells might be fused, he believes fusion events are so rare that they can't account for the extensive presence of the blue-dyed cells. A more serious limitation of the study, says Rudolf Jaenisch of the Massachusetts Institute of Technology, is that the pups were allowed to survive only through embryonic day 11—too early to tell whether the brain-derived cells were really functional. Frisén says his team is working on follow-up experiments in which the pups will develop to birth.



Optimistic skeptic. Fred Gage of the Salk Institute wants to raise the bar, but he predicts that some studies will hold up to rigorous scrutiny.

In the opinion of many scientists, the published study that comes closest to demonstrating true plasticity is by a group headed by Lagasse at StemCells, a company started by Anderson, Gage, and Weissman, that is working to develop potential applications for neural and hematopoietic stem cells. As reported in *Nature Medicine* in November 2000, the researchers purified male mouse hematopoietic stem cells based on no fewer than 13 cell-surface markers. Then they injected the cells into the bloodstream of irradiated female mice that suffered from a genetic liver disorder. The transplant not only led to the restoration of the blood systems in four of the nine mice, it also seemed to cure them of their liver disease. When the scientists killed these mice 7 months later, between a third and a half of their liver tissue was derived from donor cells.

But even this experiment falls short. Because groups of cells rather than single cells were injected into the mice, the work does not prove that the same precursor cell can generate both blood and liver cells. Grompe, one of the co-authors, also notes that the impressive percentage of liver is a bit misleading. When they looked carefully, he says, the researchers realized that the new liver tissue had grown from just a few progenitors. That's promising news for eventual medical applications, as it means that just a few cells might do the trick. But it works against the idea of plasticity, as it implies that only a very tiny proportion of blood cells managed to become liver. The team is now trying to replicate the study to rule out the possibility of fusion.



New candidate. Catherine Verfaillie has cultivated cells that can be transformed into apparent lung (*left*), duodenum (*center*), and liver cells (*right*) in young mice.

Best evidence yet?

Ironically, says Grompe, raising the bar might have a downside: Even scientists who have taken time to do careful controls might be having a harder time publishing. Exhibit A is Verfaillie, who finally has a full report of her work published online by *Nature* on 21 June. In work reported at several meetings, which earns kudos from some of the field's staunchest skeptics, Verfaillie has been examining the properties of what could conceivably be a "universal" stem cell—a type never before identified. Verfaillie grew the cells from populations of mesenchymal cells, the marrow cells that generate bone and muscle, from three species: mice, rats, and humans. Because she's not absolutely sure her cells qualify as stem cells, Verfaillie calls them multipotent adult progenitor cells (MAPCs).

Her team stumbled on these cells. "We were planning to grow mesenchymal stem cells," she explains. But "we came up with a culture system that for reasons I still don't understand appears to select for [more primitive cells]." The researchers found that after about 30 population doublings in culture, the cell population changed and took on a striking similarity to ES cells. But, at least in preliminary studies, MAPCs appear to have a big advantage over ES cells: Injecting them into live animals does not seem to cause tumors, as often happens with embryonic cells because of their mercurial nature.

Verfaillie's team has put these tiny cells—about one-third the size of mesenchymal cells—through quite a few paces, as she described at the March Keystone meeting. She has demonstrated in petri dishes that they, like mesenchymal cells, can make bone, cartilage, and fat. But unlike mesenchymal cells, they can also be cultured to produce the endothelial cells that line blood vessels, making little vascular tubes in the dish. The team has also reported (in the May issue of the *Journal of Clinical Investigation*) the creation of cells that look and function like liver cells—even producing urea. Other cells have been cultivated in vitro to show some of the characteristics of nerve cell precursors.

Going a step further than most researchers, Verfaillie has tracked the fate of individual cells. Researchers commonly use the green fluorescent protein gene to track the progeny of a group of cells. Instead, her group has used a retrovirus, which is inserted in a unique spot in every cell's genome and thus can track the specific parentage of a single cell.

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Moving beyond test tube studies, Verfaillie's team has made chimeric mice by injecting single mouse MAPCs into 12 mouse blastocysts, she reported at Keystone. This technique creates a mosaic animal—called a chimera—made up of cells derived from the original blastocyst as well as the progeny of the injected MAPCs. Four of the injected blastocysts grew into chimeras, and in two of the animals, Verfaillie reported, 45% of the body tissues tested expressed the MAPC genome. Moreover, these cells showed up in every organ, suggesting that they are capable of turning into all three embryonic germ layers: the mesoderm, the ectoderm, and the endoderm. Verfaillie doesn't yet know whether these cells will also contribute to germ line (egg and sperm) cells—a defining characteristic of ES cells.

In the final experiments Verfaillie reported at Keystone, the group infused MAPCs into young mice. The marked cells eventually showed up in lung, gut, and other tissues but were not seen in the skeleton, heart, or brain.

Verfaillie has yet to prove that her cells can fully function in the new roles they assume. For example, she observes, "we've shown they can fit into liver and make liver [products] but haven't [yet] shown they can rescue a mouse." In further experiments, she will see whether MAPCs spring into action in the heart or brain in response to injury.

Reaction to her reported work has been enthusiastic. She has shown that "the cells are stable and can contribute to a very broad spectrum of mature cell populations," says blood researcher John Dick of Toronto's Hospital for Sick Children. But her work still does not provide rock-solid evidence for plasticity, even she concedes. The missing piece, as Dick explains, is that "there's no way of knowing what the founder cell looks like"—that is, what cell gives rise to a MAPC. One possibility, says Gage, is that MAPCs are adult cells that really do show plasticity, "dedifferentiating" in culture to become multipotent. A less likely hypothesis, says Anderson, is that Verfaillie has hit upon a rare "highly multipotent" cell, a kind of universal stem cell, that could be hiding all over the body. But the fact that cells must be cultured at length before MAPCs appear "tends to argue" that they are an artifact of tissue culture, he says.

In a forthcoming paper in *Experimental Hematology*, Verfaillie describes cultivating MAPCs from mouse muscle and brain as well as bone marrow—a development that could fit with either theory. Verfaillie holds out hope that hers "could be the ultimate study that explains the results everybody else is getting."

Politicians are already keenly interested in Verfaillie's work—as a way to put ES cells out of business. Several members of Congress sought her out last winter, she says, after the press got wind of a patent application she had filed. She wrote back telling them it's too soon to draw any conclusions.

That might be a wise answer for the entire field, says Princeton's Lemischka. There are good evolutionary reasons for suppressing cell plasticity in the body. As yet, very little is known about how to change the rules while averting the dangers of running wild—a worry that applies to potential therapies derived from ES cells as well as adult cells.

So is plasticity biology's "cold fusion"? No, scientists say. Even some skeptics believe something is going on in these experiments, even if they don't know exactly what. "I think [therapies with transplanted stem cells] will eventually work," says Grompe. But "we've raised a lot of false hopes for quick fixes, and that's not going to happen." He and others say a closer comparison might be with gene therapy—greatly hyped 20 years ago but still without much to show for itself. James Thomson of the University of Wisconsin, Madison, who first isolated human ES cells back in 1998, agrees. "I'm not looking forward to the backlash 3 years from now when people say, 'What happened to stem cells?' " he says. What can scientists do about it? Says Thomson: "We need to educate the public that science takes a long time."

–CONSTANCE HOLDEN AND GRETCHEN VOGEL

CLIMATE CHANGE

Russia Can Save Kyoto, If It Can Do the Math

Russia's ratification of the Kyoto treaty might put the pact over the top. But some take a chilly view of the reliability of its greenhouse gas emission numbers

MOSCOW—The U.S. withdrawal from international negotiations over carbon emissions last year dealt a blow to the Kyoto Protocol that many thought might be fatal. A year on, however, Russia has emerged as an unlikely savior.

To come into force, the treaty must be ratified by enough industrialized nations to account for 55% of carbon emissions in 1990, Kyoto's baseline year. The U.S. withdrawal put its leading 36% share off limits, making participation by the other major players even more important. Russia—which accounts for 17% of 1990 emissions—holds second place. Its government deliberated for more than a year before President Vladimir Putin declared in April, "We'll do it." A final review of the protocol is due for completion by midsummer, with ratification expected in the fall.

What led Russia to become an environmental champion? Its economy has traditionally relied on smokestack industries and burning fossil fuels, and until recently climate change was seen benignly as an antidote for shoveling snow. But the treaty gives

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Something to hide? Russia's petroleum industry is "very shy" about revealing its emissions of greenhouse gases, including the flaring of methane from oil and gas fields.