

Reprogramming Cells

By inserting genes that turn back a cell's developmental clock, researchers are gaining insights into disease and the biology of how a cell decides its fate

THIS YEAR, SCIENTISTS ACHIEVED A LONG-SOUGHT FEAT OF CELLULAR alchemy. They took skin cells from patients suffering from a variety of diseases and reprogrammed them into stem cells. The transformed cells grow and divide in the laboratory, giving researchers new tools to study the cellular processes that underlie the patients' diseases. The achievement could also be an important step on a long path to treating diseases with a patient's own cells.

The feat rests on a genetic trick, first developed in mice and described 2 years ago, in which scientists wipe out a cell's developmental "memory," causing it to return to its pristine embryonic state and then regrow into something else. In 2008, researchers achieved another milestone in cell reprogramming. In an elegant study in live mice, they prompted cells to make the leap directly from one mature cell into another—flouting the usual rule that development of cells is a one-way street. These and other advances in tweaking cells to assume new identities add up to make the now flourishing field of cellular reprogramming *Science's* Breakthrough of the Year.

This year's breakthroughs have done much to wipe out memories of a major scandal that erupted 3 years ago, after scientists in South Korea fraudulently claimed to have used somatic cell nuclear transfer—the technique used to clone Dolly the sheep—to generate stem cells from patients suffering from type 1 diabetes, spinal cord injury, and a congenital immune disease. The debacle dealt the field a huge setback; patient-specific stem cells seemed like a distant prospect.

Breakthrough Online

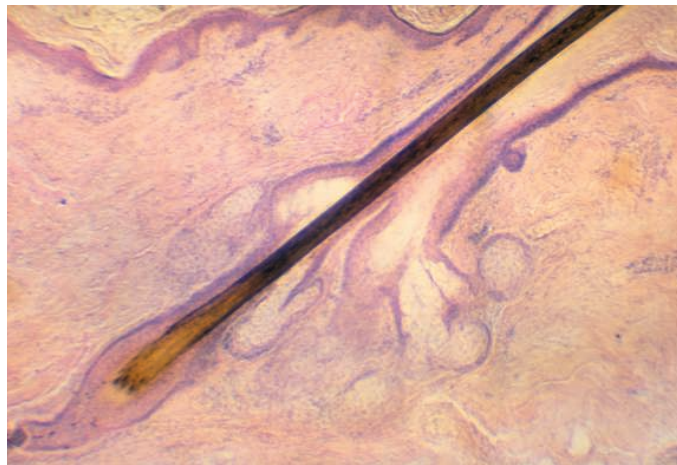
For an expanded version of this section, with references, links, and multimedia, see www.sciencemag.org/btoy2008.

The new developments build on two previous breakthroughs. Ten years ago last month, scientists in Wisconsin announced that they had cultured human embryonic stem (hES) cells—cells with the potential to form any cell type in the body. That power, known as pluripotency, opened up a world of possibilities in developmental biology and medical research, but it came with baggage: Because isolating the cells typically destroys the embryo, the research sparked fierce debates over bioethics. In many countries, including the United States, political decisions limited the work scientists could do with hES cells.

In 2006, Japanese researchers reported that they had found a possible way around the practical and ethical questions surrounding hES

Cells, made to order

For nearly a decade, stem cell biologists have sought a way to make long-lived cell lines from patients suffering from hard-to-study diseases. (Most adult cells do not survive culture conditions in the lab, so taking cells of interest directly from patients doesn't work.) This year, two groups achieved that goal. One team derived iPS cell lines from the skin cells of an 82-year-old woman suffering from amyotrophic lateral sclerosis (Lou Gehrig's disease), a degenerative disease that attacks the motor neurons, causing gradual paralysis. The scientists then directed the cells to form neurons and glia, the cells that are most affected by the disease (photos, p. 1767).



Just a week later, another group reported making patient-specific iPS cell lines for 10 different diseases (see table), among them muscular dystrophy, type 1 diabetes, and Down syndrome. Many of these diseases are difficult or impossible to study in animal models; the reprogrammed cells give scientists a new tool for studying the molecular underpinnings of disease. They may also prove useful in screens for potential drugs.

Eventually, such techniques might allow scientists to correct genetic defects in the lab dish and then treat patients with their own repaired cells.

Another paper published this year suggests that the reprogramming exit ramp does not have to lead back to an embryonic state but can take a cell directly to a new mature fate. American researchers, working in mice, reprogrammed mature pancreas cells called exocrine cells into beta cells, the cells in the pancreas that produce insulin and are destroyed by type 1 diabetes. The team injected a cocktail of three viruses into the pancreases of adult mice. The viruses primarily infected the exocrine cells, and each one carried a different gene known to play a role in beta cell development. Within days, the treated mice formed insulin-producing cells that looked and acted like bona fide beta cells.

The results are surprising because in living creatures, specialized cells almost never change course, changing, say, from a muscle cell into a lung cell. Such direct reprogramming, however, might be simpler and safer than using pluripotent cells to treat some diseases. The technique might also enable scientists to speed up the lab production of desired cell types, using defined factors to change one type of cultured cell directly into another.

Wanted: more breakthroughs

Although researchers made impressive progress in 2008, several more breakthroughs are needed before cellular reprogramming yields its first cure for disease. For reprogramming to be safe enough to use in cell therapy, researchers must find an efficient, reliable way to trigger it. They also want to understand exactly how the process works. Although dozens of labs have used the technique, what is happening inside the reprogrammed cell remains a mystery, and a combination of chance events seems to determine which rare cells end up being reprogrammed. A leading theory is that some of the reprogramming factors first help to loosen up the DNA in a cell's nucleus, making it easier to reactivate turned-off genes. Then the other factors help to set off a cascade of protein signals that give a cell its new identity (see the Review by Gurdon and Melton on p. 1811).

The original reprogramming recipe relies on viruses to insert the reprogramming genes into the infected cell's genome, altering the DNA permanently. Scientists are wary of that approach for a couple of reasons. First, the inserted DNA could interrupt existing genes—for example, those that guard against cancer, leaving the cells likely to form tumors. And although the inserted genes seem to turn off after reprogramming is finished, allowing the cell's own genes to take over, scientists worry that the inserted genes could be reactivated or could have other subtle effects on the cell.

For that reason, labs around the world are working on other ways to trigger reprogramming. This year, they made rapid progress. Several groups found that they could substitute chemicals for some of the inserted genes. Another found that adenoviruses could also do the trick, at least in mouse cells. Adenoviruses, which cause the common cold, do not insert themselves into the genome. The viruses express their genes long enough to reprogram the cells, but as the cells divide, the viruses are diluted down to undetectable levels, leaving reprogrammed cells with their original genomes unchanged. Researchers in Japan showed that rings of DNA called plasmids could also carry the required genes into the cell. The alternatives are much less efficient than the original recipe, however, and most have not yet worked in human cells, which are harder to reprogram than mouse cells.

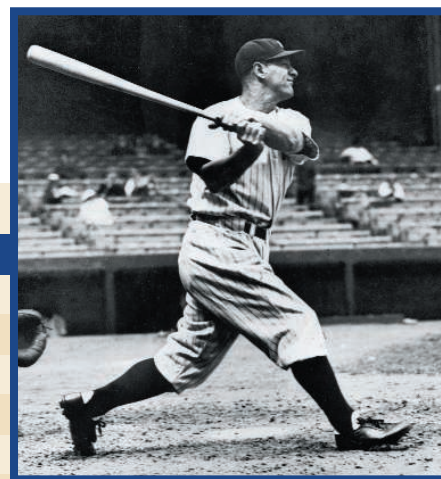
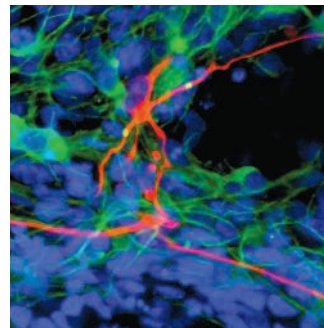
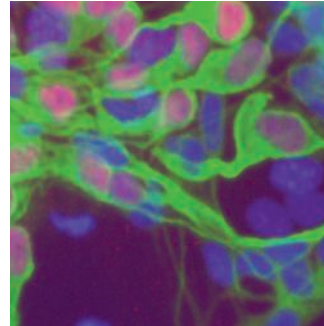
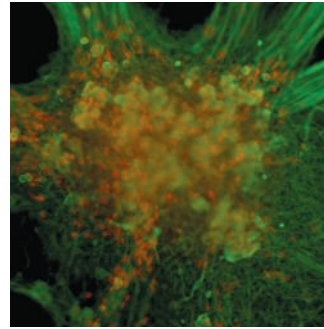
To be useful, reprogramming also needs to become much more efficient. Most experiments have managed to reprogram fewer than one in 10,000 cells. In what seems to be a lucky break for the field, however, two groups showed this year that the skin cells called keratinocytes are particularly easy to reprogram. Researchers can reprogram roughly 1%

of the keratinocytes they treat, and the process takes only 10 days instead of the several weeks that other cells require. Hair follicles (photo, p. 1766) are a rich source of keratinocytes, and researchers in California and Spain showed that they could efficiently derive personalized cell lines from cells taken from a single human hair plucked from the scalp—an even easier source of cells than cutting out a piece of skin.

Finally, reprogramming needs better quality control. This year, an American group took a major step in that direction by making cells in which the reprogramming genes could be turned on by the addition of the antibiotic doxycycline. They then used the reprogrammed cells to generate “second generation” iPS cells that are genetically identical—each contains the same viral inserts. These cells will allow scientists to study the process of reprogramming for the first time under standardized conditions and should help to reveal the biochemical processes that enable an adult cell to take an exit ramp from its one-way path of development.

A thorough understanding of reprogramming is not enough, however. Ten years after the discovery of human ES cells, scientists are still working on standardizing procedures for coaxing pluripotent cells to become mature tissue. It's a critical problem: Stray pluripotent cells used in therapies could trigger dangerous tumors. And even though scientists can easily prompt pluripotent cells to become beating heart

cells in a lab dish, no one has yet perfected a way to get such cells to integrate into the body's tissues to replace or repair their diseased counterparts. But researchers are moving faster down the highway of discovery than many had expected or dared to hope. —GRETCHEN VOGEL



Diseases With Patient-Specific iPS Cell Lines

Amiotrophic Lateral Sclerosis (Lou Gehrig's disease)

ADA-SCID

Gaucher disease type III

Duchenne muscular dystrophy

Becker muscular dystrophy

Down syndrome

Parkinson's disease

Juvenile diabetes mellitus

Shwachman-Bodian-Diamond syndrome

Huntington disease

Lesch-Nyhan syndrome (carrier)