CELLULAR REPROGRAMMING

New Technique RiPS Open Stem Cell Field

While stem cell scientists in the United States are grappling with continued uncertainty about the future of federal funding for work with embryonic cells (see p. 163), the field got a bit of good news last week: a paper describing a new method for prompting mature cells to take on a different fate. The technique is cleaner, safer, faster, and more efficient than recently developed methods for reprogramming adult cells, promising to give researchers a powerful new tool for making and using stem cells. It "will have a huge impact in the near term," says Justin Ichida, who studies reprogramming at the Harvard Stem Cell Institute and was not involved in the study.

Four years ago, scientists took a major step toward overcoming the biggest ethical hurdle in stem cell research. Instead of using cells derived from embryos, researchers found a way to make adult cells behave as though they were embryonic. Simply inserting extra copies of four genes into these cells gave the cells the ability to develop into almost any cell type in the body. Known as induced pluripotent stem (iPS) cells, these cells are a potential boon for studying and ultimately treating a variety of diseases, and many labs immediately added the technique to their repertoire. Scientists have also used the trick to turn one mature cell type directly into another (skipping the embryonic stage) by inserting key genes for the desired cell type.

But the technique, called cellular reprogramming, has some drawbacks. The reprogrammed cells retain copies of the inserted genes, which makes them prone to forming tumors and could potentially skew experimental results. And there is some evidence that iPS cells aren't exactly like embryonic stem cells in their gene expression, retaining a subtle cellular memory of the tissue they came from. The method is also relatively inefficient, reprogramming only about one out of 1000 cells exposed to the treatment, and it takes more than a month for iPS cells to appear.

The new technique goes a long way toward fixing those problems. Stem cell researcher Derrick Rossi of Harvard Medical School in Boston and his colleagues used synthetic RNA molecules that correspond to the genes inserted in classic reprogramming techniques. The technique makes iPS cells in about half the time, they reported online last week in *Cell Stem Cell*. And because the RNA quickly breaks down, the reprogrammed cells are genetically identical to the source cells.

Rossi says his first attempts to use RNA to induce protein production were stymied by cells' innate antiviral defenses, which attack foreign RNA and can trigger programmed cell death. But he and his colleagues discovered that by substituting slightly modified versions for two of RNA's usual bases, they could make synthetic RNAs that the cell accepted as its own. By inhibiting interferon—a key part of the cells' anti-RNA defense—they got the cells to express even more of the desired proteins. When the researchers applied a daily cocktail of these synthetic RNAs to connective tissue cells called fibroblasts, the cells dedifferentiated into embryoniclike cells. The team calls its cells RiPS cells, for RNA-induced pluripotent stem cells.

To the team's surprise, the process took just over 2 weeks and reprogrammed as many as 4% of the cells in the culture dish. That makes it roughly 100 times more efficient than the gene-transfer technique and twice as fast. Other researchers have been racing to find other methods to reprogram cells, but most of them have proved less efficient than the classic technique.

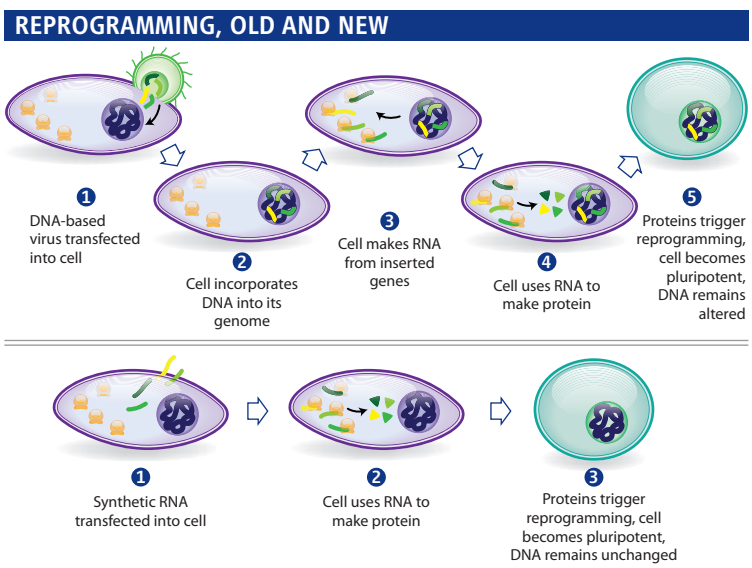
Further experiments suggest that the RNA approach does a more thorough job of reprogramming the cell than other methods. The genes that RiPS cells express are very similar to those expressed by ES cells—in other words, they seem to be a closer match to ES cells than most iPS cells to date. The method can also prompt cells to become nonembryonic cell types. By inserting synthetic RNA that codes for a key gene in muscle tissue, for example, the researchers could turn both fibroblasts and RiPS cells into muscle cells.

"I'm so impressed … that we are going to turn over our entire iPS core to this new method to make stem cells from patients with all sorts of diseases," says stem cell researcher Douglas Melton of Harvard's Stem Cell Institute. "It is a major advance."

Rossi says the synthetic RNAs will be easy for other labs to make: "If you have basic molecular biology tools, you can make these RNAs." Still, Ichida says he is not giving up his search for a suite of chemicals that can reprogram cells. For researchers who might want to make thousands of iPS cell lines, he says, RNA reprogramming will still be time-consuming and expensive, and a chemical cocktail could prove even more efficient, he says. Others agree. Although he says the RNA technique is a significant advance, stem cell researcher James Thomson of the University of Wisconsin, Madison, says it is too early to discount other approaches to reprogramming.

The RNA technique could have uses beyond the stem cell field, Rossi and others note. The modified RNA can also prompt cells to make designer proteins, Rossi says. And developmental biologists can use it to better understand the effects of certain genes. "It's the flip side of RNAi," Rossi says, referring to a widely used technique in which scientists use RNA to block the expression of genes in cells. Rossi says he will be exploring how to use the RNA-prompted protein expression to replace proteins in diseased patients. "If we could reprogram somatic human fibroblasts to pluripotency, you can do anything with this technology," he says.

–GRETCHEN VOGEL



Cleaner, safer, faster. A new technique using synthetic RNA can reprogram adult cells so they are genetically identical to the source cell.

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