



DEVELOPMENTAL BIOLOGY

Field Leaps Forward With New Stem Cell Advances

For a year and a half, stem cell researchers around the world have been racing toward a common goal: to reprogram human skin cells directly into cells that look and act like embryonic stem (ES) cells. Such a recipe would not need human embryos or oocytes to generate patient-specific stem cells—and therefore could bypass the ethical and political debates that have surrounded the field for the past decade.

The pace was set in June 2006, when Shinya Yamanaka of Kyoto University in Japan reported that his group had managed the feat in mice by inserting four genes into cells taken from their tails (*Science*, 7 July 2006, p. 27). Those genes are normally switched off after embryonic cells differentiate into the various cell types. The pace picked up in June this year, when Yamanaka and another group showed that the cells were truly pluripotent (*Science*, 8 June, p. 1404).

Now the race has ended in a tie, with an extra twist: Two groups report this week that they have reprogrammed human skin cells into so-called induced pluripotent cells (iPCs), but each uses a slightly different combination of genes. In a paper published online in *Cell* on 20 November, Yamanaka and his colleagues report that their mouse technique works with human cells as well. And in a paper published at the same time online in *Science* (www.sciencemag.org/cgi/content/abstract/1151526), James Thomson of the University of Wisconsin, Madison, and his colleagues report success in reprogramming human cells, again by inserting just four genes, but two of the genes are different from those Yamanaka uses.

Among stem cell scientists, the human cell reprogramming feats have somewhat overshadowed another major advance reported online in *Nature* last week: A team at the Oregon National Primate Research Center has officially become the first to obtain embryonic stem cells from cloned primate embryos, an advance that brings therapeutic cloning closer to reality for humans. Taken together, these feats suggest

that scientists are getting very close to uncovering the secret of just what occurs in an oocyte to turn back the clock in the DNA of a differentiated cell.

The two human reprogramming papers could help solve some of the long-standing political and ethical fights about stem cells and cloning. The technique produces pluripotent cells, cells with the potential to become any cell type in the body, without involving either embryos or oocytes—two sticking



Full of potential. Human induced pluripotent cells form teratomas, tumors with multiple cell types.

points that have made embryonic stem cell research so controversial. Ian Wilmut of the University of Edinburgh, U.K., says that once he learned of Yamanaka's mouse work, his lab set aside its plans to work on human nuclear transfer experiments, otherwise known as research cloning. The new work now confirms that decision, he says. Direct reprogramming to iPCs "is so much more practical" than nuclear transfer, he says.

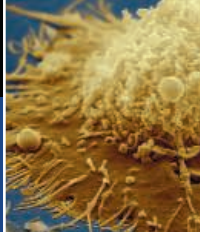
In the new work, Yamanaka and his colleagues used a retrovirus to ferry into adult cells the same four genes they had previously employed to reprogram mouse cells: *OCT3/4*, *SOX2*, *KLF4*, and *c-MYC*. They reprogrammed cells taken from the facial skin of a 36-year-old woman and from the connective tissue of a 69-year-old man. Roughly one iPC cell line was produced for every 5000 cells they treated with the technique, an efficiency that enabled them to produce several cell lines from each experiment.

Thomson says he and his colleagues already had their own list of 14 candidate reprogramming genes when Yamanaka's mouse results were published. They, like Yamanaka's group, gradually whittled down the list through a systematic process of elimination. Thomson's experiments led to four factors as well: *OCT3* and *SOX2*, as Yamanaka used, and two different genes, *NANOG* and *LIN28*. *NANOG* is another gene associated with ES cells, and *LIN28* is a factor that seems to be involved in processing messenger RNA.

Instead of cells from adults, Thomson and his team reprogrammed cells from fetal skin and from the foreskin of a newborn boy. But Thomson says they are working on experiments with older cells, which so far look promising. Their experiments reprogrammed about one in 10,000 cells. The efficiency is less than that of Yamanaka's technique, Thomson says, but is still enough to create several cell lines from a single experiment.

Comparing the two techniques might help scientists learn how the inserted genes work to turn back the developmental clock, Yamanaka says. He says his team tried using *NANOG* but saw no effect, and *LIN28* was not in their initial screen. Thomson says his team tried Yamanaka's four genes without success, but that they may have tried the wrong relative doses.

The fact that Thomson's suite doesn't include a known cancer-causing gene is a



bonus, says Wilmut. (The *c-MYC* Yamanaka used is an oncogene.) But both techniques still result in induced cells that carry multiple copies of the retroviruses used to insert the genes. Those could easily lead to mutations that might cause tumors in tissues grown from the cells. The crucial next step, everyone agrees, is to find a way to reprogram cells by switching on the genes rather than inserting new copies. "It's almost inconceivable at the pace this science is moving that we won't find a way to do this without oncogenes or retroviruses," says stem cell researcher Douglas Melton of Harvard University. "It is not hard to imagine a time when you could add small molecules that would tickle the same networks as these genes" and produce reprogrammed cells without genetic alterations, he says.

Although the cells "act just like human ES cells," Thomson says, there are some differences between the cell types. Yamanaka's group reports that overall human iPC gene expression is very similar, but not identical, to human ES cell gene expression. "It will be probably a few years before we really understand these cells as well as we understand ES cells," Thomson says. But "for drug screening, they're already terribly useful. IVF embryos are very skewed ethnically," he says. But with the new iPC technique, "you can isolate cell lines that represent the genetic diversity of the United States. And I think it will be very straightforward to do."

The primate cloning success, although partially eclipsed by the human work, "is really a breakthrough," says primate stem cell researcher Jose Cibelli of Michigan State University in East Lansing. Although scientists have cloned a host of other animals, primates have proved to be particularly resistant—as demonstrated by the failure of Korean scientist Woo Suk Hwang, whose work with human embryos was shown to be fraudulent 2 years ago.

A group headed by Shoukhrat Mitalipov was able to generate two embryonic stem cell lines after injecting skin cells from a 9-year-old male rhesus macaque into 304 eggs collected from 14 female macaques. The cells showed

all the requisite pluripotent stem cell markers; in lab dishes, they generated heart and brain neurons, and in live mice they formed teratomas—tumor tissues from all three germ layers.

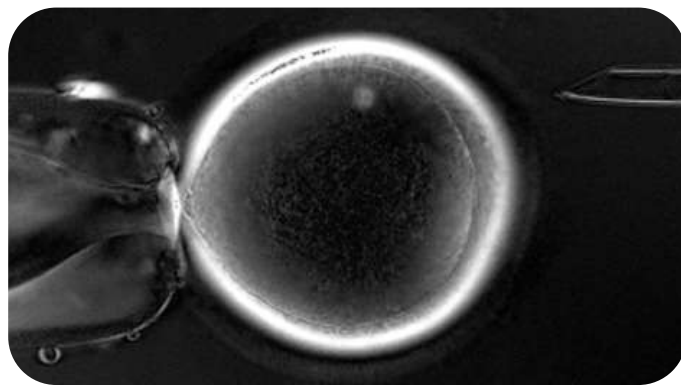
Scientists such as Robin Lovell-Badge of the U.K. Medical Research Council have lauded the feat while pointing out that the low success rate—0.7%—means more primate work is needed before women should be asked to donate eggs for such research.

Mitalipov originally reported the achievement last June in Cairns, Australia, at the meeting of the International Society for Stem Cell Research. At the time, he met with some skepticism. Before publishing the paper, *Nature* took the unprecedented step of asking a group headed by David Cram of Monash University in Clayton, Australia, to be sure the cell lines had the same genotype as the donor of the skin cells. Their report is published in the same issue of *Nature*, which issued a statement declaring this a prudent step given the importance of the results and "recent history in the cloning field."

Scientists have discovered that the big peril in cloning, as the Hwang team ultimately discovered, is that what you may really come up with are parthenotes—that is, early embryos arising solely from the activated oocyte. Parthenotes—less useful than clones because they have only the genes of the egg donors—can result when the spindle containing the nuclear DNA is not completely removed before a foreign nucleus is introduced. The usual technique for locating the spindle is with a dye or ultraviolet light, which the researchers suspected could damage fragile primate oocytes.

So instead, the Oregon group used a new non-invasive imaging system called Oosight to locate the spindle, then used a probe

to suck it out and replace it with the skin cell. Enucleation of the oocyte is 100% efficient with this technique, said Mitalipov. The scientists also changed the culture medium, eliminating calcium and magnesium, which they believe cause premature activation of the oocyte and failure of the donor nucleus to become properly "remodeled."



On target. Latest imaging technology clearly shows the egg's nucleus, to be withdrawn by pipette at right. Semos (*below*), the male macaque whose skin cells made history.

Although the cloning "efficiency is still low," Mitalipov said at a press conference, "I believe the technology we developed can be directly applicable to humans."

Robert Lanza of Advanced Cell Technology in Worcester, Massachusetts, calls the Oregon paper a "turnaround," saying that it marks a "recovery for the field," because the Hwang paper was retracted in January 2006. The next step, says Mitalipov, will be to test cloning for treatment of a disease, something that hitherto has been tried only in the mouse. A likely target is diabetes, says Mitalipov, who plans to inject cloned, genetically modified ES cells into a monkey model of the disease.

"I cannot emphasize enough how useful these [cloned primate ES] cells will be" for studying other diseases that also affect humans, says Cibelli. Another application, he says, will be to compare the cloned primate ES cells with cells reprogrammed by the methods Yamanaka and Thomson used. "If their method is as good as the oocyte" in reprogramming somatic cells, says Cibelli, "we will be no longer in need of oocytes, and the whole field is going to completely change. People working on ethics will have to find something new to worry about."

—GRETCHEN VOGEL AND CONSTANCE HOLDEN

