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## Stem Cells Recall Their Origins

Cells, it turns out, remember where they came from. Four years ago, scientists made a breakthrough in stem cell research, when they discovered how to turn back the developmental clock on skin cells, muscle cells, and other “adult” cells so the cells would behave like embryonic stem cells. These induced pluripotent stem cells (iPS cells) were touted as an alternative to the ethically contentious embryonic stem cells.

Now, though, two groups of Howard Hughes Medical Institute researchers report that iPS cells retain a genetic memory of their tissue of origin. In a sense, the iPS cells “remember” that they came from skin, muscle, blood, and so on. This memory impedes the transformation of iPS cells into other types of cells, a prospect that has deep implications for researchers working with these kinds of cells, say HHMI investigator George Q. Daley and HHMI early career scientist Konrad Hochedlinger, who led the two research groups. The scientists worked independently but shared manuscripts and coordinated joint publications on July 19, 2010 in *Nature* (Daley) and *Nature Biotechnology* (Hochedlinger).

Creating iPS cells is an important research tool because the technique can be used to generate disease-specific stem cell lines that, like embryonic stem cells, can develop into many cell types.

“The backdrop to this research is that a lot of people have the impression that iPS cells are the equivalent of embryonic stem cells,” says Daley. “That has been used as an argument that we do not need to keep studying embryonic stem cells. But iPS cells often don’t function as well as embryonic cells, and our new research offers an explanation as to why that is the case.”

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Daley and his colleague Kitai Kim stumbled onto the iPS memory phenomenon while trying to cure mice of the blood disease thalassemia. Thalassemias are caused by inadequate production of the globin protein, which helps transport oxygen in red blood cells. The disease can be caused by different mutations in globin genes that reduce the blood cells' ability to carry oxygen. In investigating a possible treatment model for human disease, Daley's group took skin cells from a sick mouse, turned those cells into iPS cells, repaired the defective gene that causes thalassemia, and then attempted to grow healthy blood cells from the iPS cells to implant back into the mice. But the iPS cells failed to make many blood cells.

Puzzled, the team then repeated the procedure, this time starting with blood cells instead of skin cells. These "blood-originated" iPS cells made many more blood cells than the iPS cells that had begun as skin cells. "This was such a fascinating observation we really wanted to understand it," says Daley, who is director of the Stem Cell Transplantation Program at Children's Hospital Boston.

Working with colleagues at Harvard Medical School and Johns Hopkins University, the team analyzed gene activity in iPS cells that had originated from different tissues. In particular, they scanned the genomes of the iPS cells for a chemical signature indicative of gene silencing. This chemical signature, called methylation, tells the cell to deactivate the expression of certain genes. New technology allowed the team to scan nearly all of the methylation sites across the entire genome of the iPS cells – in essence, surveying which genes were switched off. Hochedlinger's team performed a similar analysis.

Both teams found that iPS cells that originated from different tissues displayed different gene activation and silencing patterns. In iPS cells that had originated as skin cells, for instance, genes required for blood cell formation were silenced. Likewise, iPS cells originating as blood cells had silenced the genes required to make bone cells. "The differences we found were significant enough that we could distinguish the iPS cells from different tissues," says Hochedlinger, an associate professor of stem cell and regenerative biology at Harvard University.

Hochedlinger adds that researchers need to be aware of these differences when using iPS cells to study human diseases. For instance, if a researcher is studying neurons grown from iPS cells isolated from patients with Parkinson's disease, those cells may behave differently depending on whether the iPS cells originated as skin cells, muscle cells, or brain cells. "Any subtle differences you see in your patient-derived iPS cells could in fact be the result of not only the disease abnormality, but also the memory retained in the iPS cells," he says.

Daley says another implication is obvious: If a researcher wants to study, for example, a blood disease with iPS cells, it makes sense to begin with blood cells rather than skin cells, which have become the standard starting cell in

iPS experiments because they are easy to retrieve.

However, both teams offer intriguing footnotes—methods for erasing the memory of iPS cells to make the cells more like embryonic stem cells. Daley's group found that drugs that modify DNA methylation could reset iPS cells into a more embryonic state. In practice, that means those cells more easily formed all of the tissues of the body than iPS cells not treated with the drugs. Hochedlinger, in contrast, found that simply growing iPS cells in dishes for a long period – about three weeks – erased the memory of those cells' origins. "The longer you passage the iPS cells, the closer you come to getting embryonic stem cells," he says. "Reprogramming the cells seems to be a slow process."

Both groups conducted their experiments in mouse cells, but they expect the results to hold in human iPS cells, too. Hochedlinger has begun confirming the results in human iPS cells.