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## Cells Induced to De-Differentiate Back into Stem Cells

For the first time, researchers have induced differentiating cells to revert to being stem cells. Although such de-differentiation is known to occur in natural systems, scientists had never before mimicked the process in the laboratory. The researchers said their achievement with the fruit fly *Drosophila* suggests that de-differentiation should be explored as yet another route to generating stem cells for therapeutic purposes.

Stem cells—isolated from embryos or from adult tissue—are immature progenitor cells with the capability to differentiate into a variety of specialized cells that form tissues and organs. Scientists are working toward using stem cells to grow mature specialized cells that could regenerate damaged or diseased tissues of the brain, heart or other organs.

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— Allan C. Spradling

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The researchers, Howard Hughes Medical Institute investigator Allan Spradling and colleague Toshie Kai, reported their findings in the March 14, 2004, advanced online edition of the journal *Nature*. Both are at the Carnegie Institution of Washington.

According to Spradling, it has long been known that some specialized, or differentiated, cells in the body can revert to become stem cells to regenerate tissue under particular conditions. "One of the classic systems is the mammalian liver, in which if you remove pieces of the organ, whole lobes of the liver can regenerate with all their intricate structure," he said. "And surprisingly, there seems to be a major contribution to this regeneration from cells that are already differentiated liver cells, not just a reserve population of stem cells."

“The fact that cells of that type can differentiate to produce a highly organized tissue suggests that de-differentiation is a possible source of progenitor cells,” said Spradling.

The problem in studying the mechanisms of such de-differentiation in many stem cell systems, he said, is that their organization, behavior, and regulatory mechanisms have not been characterized well enough at the level of individual cells. However, the location, movement and molecules regulating individual germline stem cells, are characterized well enough known in *Drosophila* to make such studies feasible.

To induce and study de-differentiation, Kai and Spradling manipulated cells in the ovary of the female fruit fly larva. In normal egg development in the adult fly, germline stem cells reside in a microenvironment, called a niche, before they are called on to differentiate into eggs, or oocytes. When differentiation is triggered by a regulatory protein called Bam, the stem cell forms a cyst that ultimately develops into an egg.

However, in the larva, the niche has yet to form and the stem cell progenitors remain undifferentiated. To observe de-differentiation, the researchers introduced an engineered form of the bam gene that produced brief bursts of the Bam protein when the larvae was subjected to a mild heat shock. This brief exposure to Bam caused the stem cells to begin to differentiate.

“However, the Bam protein only lasts for about twenty hours, and then disappears,” said Spradling. “But unlike the adult ovary, in the larval ovary, the cysts made in this way are unstable after Bam disappears, and we can see them break down and the cells de-differentiate. Moreover, all the germ cells in the ovary undergo both the formation and breakdown process, uniformly and without detectable cell loss.

The researchers confirmed that the differentiated germ cells had reverted to stem cells, because once the heat-shocked larvae matured, their stem cells, which derived entirely from the reverted cells, functioned normally and the adult flies were fertile.

According to Spradling, the confirmation of stem cell de-differentiation suggests the importance of focusing on this mechanism as a source of stem cells. “There's a great deal of excitement about the potential of embryonic stem cells, and I think rightly so,” he said. “I wholeheartedly support that work. However, it is a fairly challenging route to go from what is a very undifferentiated early embryonic stem cell all the way through differentiation to an adult tissue cell. Thus, it could also be fruitful to focus on the reverse route.

“We know that in natural systems, differentiated cells can go back to a progenitor state,” he said. “But I don't know of many cases where cells go from an embryonic state all the way to a progenitor state in an adult organism. One might imagine it would be a lot easier just to go back to a progenitor state of the same lineage, than to go all the way from the embryonic state.”

However, said Spradling, the leap from germ cells to other cell types may be a considerable one. “It may be that downstream of every stem cell, the rate at which cells differentiate differs,” he said. “It might be that the cells we studied are particularly slow in changing their gene expression or reprogramming their chromosomes. And because they haven't done very much at the cyst stage, and most of the changes are in the cytoplasm, it may be relatively easy for them to go back. However, these *Drosophila* studies are still encouraging, because at least they demonstrate that you can revert a cell that looks morphologically distinct from a stem cell, and at an extremely high efficiency,” he said.

In future studies, Spradling and his colleagues plan to explore in detail the genetic and molecular changes underlying de-differentiation in the ovarian cells. To that end, they reported in the *Nature* paper that they had induced de-differentiation on a much larger scale in adult flies.