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## Giving Cells a Fresh Start

Howard Hughes Medical Institute (HHMI) researchers and their colleagues have identified an enzyme that can effectively wipe a cell's developmental slate clean, essentially giving a fresh start. The enzyme, which is thought to help genetically reprogram fertilized eggs as part of normal development, may help scientists create stem cells and arrest the growth of cancers.

The new research, reported in an online article in the journal *Nature* on January 6, 2010, represents a collaborative effort of scientists from the laboratories of HHMI investigator Yi Zhang at the University of North Carolina, Chapel Hill, and Teruhiko Wakayama at the Center for Developmental Biology in Kobe, Japan. Coauthors of the article are Yuki Okada and Kwonho Hong, postdoctoral researchers in Zhang's lab, and Kazuo Yamagata of the Wakayama lab.

During growth and development, genes that should not be expressed are physically tagged with chemicals, such as methyl groups. These chemical modifications are epigenetic, meaning they influence the expression of genes, but they are not part of the actual gene sequence. The addition of a methyl group to a DNA molecule inactivates a gene, whereas demethylation—subtraction of a methyl group—activates the gene.

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Scientists had long hypothesized that somewhere inside the cell there might exist a DNA demethylase enzyme that would be able to "genetically reformat" cells by wiping off all their methylation tags. Such an enzyme might be necessary, for example, to help guide an egg's transformation into an embryo. When a sperm enters an egg cell during fertilization, almost all of the methylation marks on its DNA are suddenly erased. A similar epigenetic stripping seems to occur when an egg cell is fused with another cell in cloning and stem cell experiments done in the laboratory.

“If you want to make a big change in a cell’s fate, you need something that can cause global demethylation of its DNA,” Zhang said. “That’s why finding an enzyme that naturally performs this task has been one of the major goals of developmental biologists in recent years.”

A general-purpose DNA demethylator might make it easier for researchers to generate stem cells from somatic cells in the body, which could be used for research or therapeutic purposes. “Incomplete demethylation of cellular DNA appears to be one of the major bottlenecks causing a low efficiency in stem cell reprogramming these days,” Zhang said. “If you could add a global demethylase, you might be able to speed up the reprogramming process.”

Such a demethylase might be useful in cancer therapy too, because there is evidence that some cancers develop only after tumor-suppressor genes have been methylated, and thus silenced. “If you could somehow reactivate those silenced tumor suppressor genes by causing them to lose their methylation, then the cancer cell could become a more normal cell again,” Zhang said.

Zhang and his colleagues began their search for a DNA demethylase by screening a series of mouse genes that meet two major criteria: First, they must have a DNA sequence that suggested they would code for an enzyme-like protein. Second, the gene’s expression level in egg cells had to rise sharply after fertilization, making it more likely that it had some role in embryonic development. The researchers then used an RNA interference technique to “knock down” the expression levels of each candidate gene and measured how each gene knockdown affected paternal-DNA demethylation.

Six candidate genes met the criteria established by Zhang and his colleagues. Knocking down one of these genes, *Elp3*, resulted in a major reduction in demethylation of sperm DNA. With further experiments, Zhang and his colleagues were able to attribute *Elp3*’s effects on demethylation to a specific region of the protein that is similar to a group of enzymes known as “radical SAM” enzymes -- so-called because they use the molecule S-adenosylmethionine (SAM) to catalyze reactions involving free radicals. When Zhang and his team blocked only this portion of the enzyme, they found that it reduced demethylation about as sharply as if all of *Elp3* had been blocked.

Intriguingly, the knockdown of two genes known to function together with *Elp3* -- *Elp1* and *Elp4* -- also impaired DNA demethylation in sperm. *Elp1*, *Elp3*, and *Elp4*, along with three other *Elp* genes, code for proteins that form Elongator, a molecular complex with multiple functions. Many of Elongator’s functions remain mysterious, Zhang said, and it is plausible that demethylating DNA is one of its roles. In further tests, he and his colleagues found that the expression levels of *Elp* genes jump several-fold just before demethylation of the paternal genome occurs.

Zhang hypothesizes that the radical-SAM domain of the Elp3 protein might initiate a reaction that leads to removal of a methyl group from DNA. “But from the data we have published so far, we cannot conclude that it’s the enzyme,” he said. So he and his team are following up with experiments to prove that Elongator/Elp3 really is the long-sought DNA demethylase. One way to prove that would be to show biochemically that Elp3 or the Elongator complex can catalyze demethylation in vitro. Another would be to show that deleting Elongator or its key domains prevents paternal genome demethylation in fertilized eggs (also called zygotes). “We’ll see whether we can get a mature egg from Elp3-knockout mice, and if so, whether the demethylation still occurs in the Elp3-deficient zygotes” Zhang said.