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Gene Keeps Neural Cells on Correct Developmental Path

Embryonic stem cells with identical genomes grow into distinctive tissues, such as heart, bone, and brain. At one time, scientists believed the differences among cell types arose from various sets of genes switched on inside developing cells. Then, studies showed that adult neurons uniquely lack a protein that permanently turns off neuronal genes in the rest of the body's cells.

Now, it turns out that precursor nerve cells contain that same repressive protein after all. In fact, the protein directs the complex network of genes that transforms an embryonic stem cell into a mature nerve cell, say Howard Hughes Medical Institute (HHMI) researchers.

This new study, published in the May 20, 2005, issue of the journal *Cell*, may be among the first to track a set of genes from stem cell to differentiated neuron. It also reveals fundamental details of how stem cells retain developmental plasticity.

"A single protein does it all," said Gail Mandel, HHMI investigator at the State University of New York at Stony Brook. "It keeps the genes totally off in non-neuronal tissues, such as skin, where you don't dare express a neuronal gene. But it also allows the full elaboration of the neuronal phenotype from the precursor cell."

Led by HHMI associate Nurit Ballas, a postdoctoral fellow in Mandel's lab, the study may advance stem cell research aimed at understanding repairing spinal cord injuries or replacing malfunctioning brain cells in neurodegenerative diseases. It may also provide insights into other diseases, such as small cell lung cancer, which mistakenly make neuronal proteins, or neurological syndromes, where neuronal proteins produced by cancers may trigger the immune cells to attack the nervous system.

The study focuses on a protein called REST, which is short for RE1-silencing transcription factor. It was independently discovered 10 years ago by Mandel's group and a second team led by HHMI investigator David J. Anderson at Caltech. Mandel created the acronym to describe how REST quiets the nerve genes. The protein is also known by the name Anderson gave it, NRSF, for neuron-restrictive silencer factor.

Since then, they and others have found that REST locks down neuronal genes in other cells by grabbing onto the DNA and cementing in other molecules, an arrangement that stays intact as non-neuronal cells differentiate into liver, muscle, and other tissues.

The new study reports that REST uses a different temporary off mechanism to direct neuronal development. "This study shows that there is more than one way to keep a REST-regulated gene repressed," said Michael G. Rosenfeld, an HHMI investigator at University of California, San Diego, who co-authored an accompanying commentary in *Cell* with Victoria Lunyak, a research associate in his lab.

In contrast to the tight packaging of neural genes in other cells, REST keeps the chromatin in embryonic stem and precursor neurons open and poised for gene activity.

"REST keeps the brake on lightly until a trigger tells embryonic stem cells it's time to make a neuron," Mandel said. The cell then triggers the expression of an ensemble of genes that coordinates nervous system development by removing REST in three distinct phases, ending with shutting down the REST gene.

"The cell gets rid of all the excess protein, kicks it off the DNA, then stomps on its head so it can't make RNA," Mandel said. "We can't detect REST in the terminally differentiated neuron." But some molecular partners of REST remain, perhaps fine-tuning gene expression in mature neurons, she said.

REST seems to work globally, binding to the starting points of as many as 1,000 genes at once. The gradual loss of REST in differentiating neurons probably orchestrates a precise sequence of genes sensitive to different levels of REST, Mandel speculates.

REST has been a difficult gene to study. Using knockout technology — a popular technique for determining gene function — does not work for REST because mice lacking the gene die before they are born. Embryonic stem cells provided a way for Mandel to get around this problem. Unexpectedly, they also revealed fundamental ways in which stem cells remain plastic.

"This paper is like a whole story, beginning with the birth of a neuron and ending with the death of REST," Mandel said.