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Tinkering With the Clockwork of Developing Neurons

Howard Hughes Medical Institute (HHMI) researchers have developed a method for manipulating the molecular “ticks” of the regulatory clock that governs the development of neurons in fruit flies.

Understanding how to reset the developmental clock could prove useful in stem cell research where restoring the full potential of older cells could lead to new treatments for a variety of diseases.

HHMI investigator [Chris Doe](#) and colleague Bret Pearson at the University of Oregon reported their findings in the October 9, 2003, issue of the journal *Nature*.

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- Chris Q. Doe

“It’s been accepted for the past century that cells change over time as they go through development and divisions,” said Doe. “But nobody has had any idea about the molecular mechanisms that underlie those changes. In the case of stem cell research, it would be extraordinarily useful to know how these precursor cells change over time so that we could reset them back to the beginning where they have their full potential,” he said.

According to Doe, precursor neuronal cells, or neuroblasts, of the fruit fly *Drosophila*, offer a well-defined model for investigating the machinery that regulates neuronal differentiation.

“In studying mice, for example, it’s only possible to look at a population of neural precursor cells, but not to follow the development of individual cells,” said Doe. “In contrast, in *Drosophila*, each precursor is identified and always

generates the same family of neurons; this makes it easier to study. *Drosophila* neuroblasts exhibit many of the features of mammalian neural stem cells, so we also hope there will be similarities in the mechanisms used to regulate changes that precursors go through over time.”

In their experiments, Pearson and Doe chose to follow the development of a particular neuroblast, named NB7-1, because they knew that the Hunchback protein influenced the development of this type of neuroblast. Doe and his colleagues had previously shown that Hunchback could stop the developmental clock of neuroblasts by maintaining the cells at an early stage in development.

Hunchback is a “transcription factor”—a master genetic regulator in the cell that controls the activity of collections of genes. Neuroblasts normally express the Hunchback protein only when young. Hunchback expression disappears as neuroblasts progress to later developmental stages and come under the influence of other transcription factors.

At each of these developmental stages, the neuroblast gives rise to specialized types of neurons that make up the fly's brain and nervous system.

“We asked the question, ‘What if we took an old neuroblast and gave it the Hunchback transcription factor?’” said Doe. “Could we revert it back to the young state?”

To answer that question, the researchers used molecular techniques that enabled them to reactivate the Hunchback protein permanently in progressively older NB7-1 cells, as well as to produce abrupt bursts of Hunchback at will.

“We found that if we kept Hunchback on in the neuroblast much longer than normal, then switched it off, the neuroblast would proceed to make its succession of normal progeny,” said Doe. “That told us that the cells retained their normality despite the manipulation. It also gave us the exciting result that neuroblasts can be maintained in a “young” state for a long time without losing their potential to make their normal family of neurons, including the later-born ones. All we needed to do was switch off Hunchback.

“Then we did experiments in which we gave a succession of pulses of Hunchback at older and older ages. We demonstrated that as the neuroblasts aged, they lost the ability to make those young neurons—that the neuroblast was changing over time.”

The mechanism underlying such changes is known as “progressive restriction,” and according to Doe, the experiments with NB7-1 and Hunchback represent the beginning of a major effort to explore how that mechanism works. “For the first time, we have an experimental system to study progressive restriction in a single neural precursor using molecular

genetic techniques,” he said.

For example, said Doe, one leading theory suggests that regulatory proteins such as Hunchback act as silencers of genes for transcription factors that trigger later development; and the disappearance of Hunchback allows those genes to switch on. By keeping Hunchback on, neuroblasts could maintain their full potential by preventing the expression of genes that induce differentiation of later-born cell types.

Further studies, he said, are concentrating on searching for mutations in the fly in which progressive restriction is either reduced or enhanced. By identifying the genes that are mutated, said Doe, researchers may discover the molecular mechanism that regulates neuroblast aging, and gain insight into how to keep fruit fly, and perhaps mammalian, neural stem cells young.