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How Brain Cells "Remember" Their Birth Order

While teasing out the molecular signals that govern neural development in fruit flies, researchers have discovered how brain cells "remember" the order in which they are "born" from precursor stem cells. This type of molecular memory appears to determine the specific cell type the newly born cells will become and influences where in the developing brain those cells will reside permanently.

In an article published in the August 24, 2001, issue of the journal *Cell*, Howard Hughes Medical Institute investigator Chris Q. Doe and colleagues at the University of Oregon reported that *Drosophila* neural precursor cells, called neuroblasts, sequentially activate four different transcription factors. Transcription factors are proteins that activate or repress the expression of genes.

This sequence of transcription factor activation allows the neuroblasts to give rise to a series of different daughter cells, which ultimately become neurons and glial cells in the fruit fly brain. The scientists found that the daughter cells continue to produce the particular transcription factor that was active in the neuroblast at the time of their birth - a "memory" that allows neurons to maintain differences based on their time of birth. For example, first-born neurons always make the longest axon projections to distant targets, compared to later-born neurons from the same stem cell.

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- Chris Q. Doe

"Before we started this work, it was known that each neuroblast makes many different types of neurons and glia, and that occurs in a particular order," said

Doe. "But the genes that control the fate of the cells in that sequence were not known. Also, it was not known whether there is one set of genes that works for all stem cells, or whether each of the many different types of stem cells in the fly has its own set of genes that work in some combination."

According to Doe, *Drosophila* is an ideal model for studying the order in which specific neural cells arise from stem cells because there are well known markers that can be used to determine the stage of development at which individual neurons are born. "So we can search for the molecular markers -- such as transcription factors, neurotransmitters and other gene products -- that reveal early, middle and late neurons," said Doe. "We can also create and study mutants in genes that affect this birth order."

In their experiments, the scientists sought to understand in what order the fly neuroblasts expressed four transcription factors that were good candidates for regulating the birth order of daughter cells. These transcription factor genes are called *hunchback*, *Krüppel*, *pdm* and *castor*.

"We first established when each neuron was born from a particular stem cell," said Doe. "And once we had that foundation, we looked for genes that correlated with the birth of the first-born cells, second-born, third-born, and so on. And then we manipulated those cells genetically to determine whether the transcription factors made any difference in the kinds of neuronal cells they would become."

The experiments revealed that the neuroblasts sequentially and transiently expressed *hunchback*, *Krüppel*, *pdm* and then *castor* -- in that order. "We found these windows of expression in which a neuroblast would express just one of the transcription factors," said Doe. "Then the daughter cells born during that window - say, the window during which *hunchback* was being expressed - would maintain *hunchback* expression permanently. And that's one way the daughter cells can have a molecular memory of their birth order."

This order of genes is critical for normal brain development, because when one of the first genes in the sequence (*hunchback*, for example) is artificially turned 'on' continuously in a stem cell, that cell will repeatedly make first-born neurons and never make the later-born neurons that it would normally, Doe explained. Thus, the sequential expression of all four genes is necessary to make different neurons from a single stem cell.

"The bottom line is that we have found four genes that work in a concerted way in every stem cell lineage," said Doe. "So, it's not that each stem cell has its own set of genes, but rather all of the *Drosophila* stem cells that we've been working on use that same set of four genes."

According to Doe, while fruit flies are evolutionarily distant from mammals, the lessons learned about neural development in the insect might well be important in understanding human neural development. "The *Drosophila* system is simple compared to mammals, but what I have found amazing is the apparent parallels between mammalian cortical development and neural stem cells in flies. Just as in flies, there are precursor cells in the mammalian central nervous system that sequentially produce the different types of neurons that go to the different cortical different layers."

Doe emphasized that researchers still do not know whether flies and mammals use the same kinds of transcription factors to control neural development. However, he and his colleagues are currently exploring possible parallels in neural development between flies and mammals.

According to Doe, if the transcription factors are similar, findings in flies could have important implications for the clinical use of human neural stem cells to regenerate brain tissue damaged by such disorders as Parkinson's disease.

"If stem cells undergo an irrevocable change as they go through this sequence of gene expression, they will not be very useful for therapeutic purposes, unless we can learn to reset them back to 'ground zero,'" said Doe. "Or, if we can learn to change them by re-expressing one or another of the transcription factors, we can essentially reset them so they can be used to replace particular kinds of neurons lost to disease," he said.