STEM CELLS

When is a stem cell specified?

Using time-lapse imaging, researchers study the potency and fate specification of live neural stem cells in culture.

"One of the most interesting things that stem cells do," says Ron McKay at the National Institutes of Health, "is that they change into other cells. And if we want to know how they pull this off, we have to have a precise measurement of when those changes are occurring."

In recently published work, McKay and his colleagues do just that. Using a combination of time-lapse live-cell imaging and retrospective fate mapping, they established the lineage history of cultured multipotent neural stem cells derived from the brain of the embryonic rat.

The researchers built a special live-cell culture chamber, allowing control of gas and medium exchange, as well as of temperature and flow rates, to image these cells over time. "These are not HeLa cells," McKay emphasizes. "They are sensitive to small perturbations; even taking them out of the incubator has big effects."

With this culture chamber, and using etched growth areas to restrict cell migration out of the microscopic field of view, McKay and colleagues were able to watch the neural stem cells divide and differentiate over 6–8 days. They manually determined the lineage—the pattern of cell divisions over time—from the time-lapse images. And at the end of the experiment they used immunostaining and morphology to establish whether the cells present had neuronal, astrocytic or oligodendrocytic identity, the three cell types that neural stem cells typically generate.

With this information in hand, the researchers could then identify statistically which cells in the lineage were 'specified', meaning that they produced other cells of only one type. "One of the really interesting conclusions of this study," says McKay, "is that the cells become specified very early, days before fate-specific markers are expressed. I think we really need to look at this and ask what the mechanism is."

The researchers identified tripotent, bipotent and unipotent (that is, specified) cells, and monitored reproducible and nonrandom shifts in these populations over time. What is more, treatment with ciliary neurotrophic factor (CNTF), known to promote astrocytic differentiation, changed the distribution of these populations compared to the control. As expected, CNTF treatment increased the number of astrocytes and reduced the number of neurons and oligodendrocytes. But notably, it promoted astrocytic identity in multiple ways. CNTF not only promoted proliferation of specified astrocytic precursors but also increased the number of transitions from tripotent to unipotent astrocytic cells, bypassing two of the bipotent states seen under control conditions.

McKay and colleagues also noted a high degree of heterogeneity in the cultures, even at early stages in the *in vitro* lineage, when tripotent cells are most common. "It's almost as if they are stochastically multipotent stem cells," says McKay.

Studying stem cell lineages will generate datasets that are informative about the inherent abilities of these cells to execute developmental transitions in culture. An obvious potential concern, however, is the limitation of such a system to cells *in vitro*. "One needs to be nuanced about it," points out McKay. "We're not saying that this exact cell exists in the body. And of course there are going to be a whole lot of other factors that play a role *in vivo*."

But the technical challenges to carrying out such a study in an animal are formidable, if not insurmountable at present. And although lineaging *in vitro* will not replace studies in animals, it may, with its superior resolution and precision, have a lot to teach about the molecular mechanisms underlying specification of cell fate.

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RESEARCH PAPERS

Ravin, R. *et al*. Potency and fate specification in CNS stem cell populations *in vitro*. *Cell Stem Cell* **3**, 670–680 (2008).

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