

Sequencing Single Cells Reveals Sequential Stem Cell States

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Understanding the molecular mechanisms underlying activation of quiescent neural stem cells (NSCs) is complicated by heterogeneity in coexisting NSC pools. Two papers in this issue of *Cell Stem Cell* (Llorens-Bobadilla et al., 2015; Shin et al., 2015) report sequencing of single NSCs, providing insights into the transition from quiescence to activation and highlighting common themes in NSCs from distinct brain regions.

Neurogenesis in the adult rodent brain occurs continually in two regions, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus, where radial glia-like cells persist into adult and retain the capacity to divide and differentiate into mature neurons and glia (Aimone et al., 2014). In both of these regions, however, the populations of neural stem cells (NSCs) responsible for these activities are thought to be heterogeneous. Recent work has suggested the potential coexistence of distinct NSC pools that may differ in expression of marker sets and divergent capacity for generating daughter cells, as well as relative states of quiescence and activation (Mich et al., 2014). The relationship between NSCs in these different states and how transitions between them are molecularly controlled, and how that may relate to apparent functional differences in contributions to tissue homeostasis and response to injury, remains unclear. Now, two studies in this issue of *Cell Stem Cell* focus on the neurogenic SVZ and SGZ regions and use the power of recently developed single-cell methods to dissect the diversity and lineage trajectories of stem cells in these two regions (Llorens-Bobadilla et al., 2015; Shin et al., 2015).

From the first demonstrations that the RNA of single cells could be sequenced (Tang et al., 2009) through a series of technical improvements (reviewed in Shapiro et al., 2013), it is now possible to analyze the RNA of thousands of single cells, at low cost, with near-perfect quantitative accuracy and reasonably good sensitivity. These technical improvements

have also been translated into biological understanding and have successfully been used to reconstruct cellular lineages in several tissues, for example lung epithelium (Treutlein et al., 2014), and to classify neuronal and non-neuronal cell types of the cerebral cortex (Zeisel et al., 2015). Although both Llorens-Bobadilla et al. and Shin et al. used markers to isolate prospective NSCs from surrounding cells, both of these studies capitalize on these advances to gain a detailed molecular understanding of single NSCs, providing insights into lineage relationships and molecular regulators underlying NSC activation.

Llorens-Bobadilla et al. have focused on the SVZ and used markers to isolate putative quiescent and active NSCs as well as neuroblasts (that is, immature cells committed to become neurons). They subjected each individual cell to single-cell RNA sequencing and then used clustering to identify distinct cell types or states. They identify four substates of NSCs, which they link into a proposed differentiation trajectory using the concept of “pseudotime” and the Monocle algorithm (Trapnell et al., 2014). Pseudotime is a trajectory through the high-dimensional gene expression space, along which cells progress as they differentiate and mature. Monocle discovers a pseudotime trajectory by reducing overall dimensionality using Independent Component Analysis (ICA), constructing a minimum spanning tree through this reduced space, and finally, locating the longest path, which is assumed to capture the extremes of the differentiation process. The trajectory

in this case suggested a gradual transition from a quiescent stem cell through a “primed quiescent” stage and into actively dividing states. In agreement with this, it was found that upon ischemic brain injury, the quiescent cells were nearly absent, whereas there was an enrichment of cells in primed quiescent and active states. These findings suggest that quiescent stem cells detect injury signals, which cause them to transition to a primed state and subsequently become actively dividing stem cells. The existence of a primed quiescent sub-state is, as the authors suggest, reminiscent of the mTORC1-controlled G_0 to G_{Alert} transition observed in muscle stem cells (Rodgers et al., 2014), although it is unclear if the molecular mechanism is the same or merely analogous. In either case, an improved understanding of the mechanisms that activate quiescent stem cells in adult tissues can guide efforts to discover drugs that help repair injured and aged organs.

The second study, by Shin et al., focuses on the SGZ of the dentate gyrus. The authors used a CFP reporter under the Nestin enhancer to isolate putative NSCs. The cells were subjected to RNA-seq and arranged in pseudotime order. The authors developed a novel pseudotime algorithm called Waterfall. Similar to Monocle, Waterfall creates a pseudotime trajectory by calculating a minimum spanning tree on a reduced representation of the expression data, which essentially creates a chain of similar cells. Waterfall extends Monocle by then using a hidden Markov model (HMM) to infer changes in

gene expression along this trajectory, which enables the discovery of lineage-regulated genes. The authors used Waterfall to reconstruct the molecular events that occur when quiescent stem cells activate, divide, differentiate, and mature. Crucially, as in the study by Llorens-Bobadilla et al., the use of single-cell RNA-seq ensures that every step of this process is associated with a complete transcriptome profile, often from many single-cell replicates. In other words, single-cell analysis provides access to the temporal dynamics of the adult NSC lineage, in glorious whole-transcriptome detail, from a single snapshot of the tissue.

Interestingly, in both studies quiescent NSCs were characterized by the expression of a set of genes that were almost indistinguishable from those also specifically expressed in parenchymal astrocytes. For example, the transcription factors *Sox9*, *Id2*, *Id3*, and *Id4*, here identified as specific to quiescent NSCs, are all also expressed in astrocytes. Only a handful of genes were found to be specific to quiescent NSCs in either the SVZ or the SGZ. This speaks to the close relationship between quiescent NSCs and astrocytes, which both are the offspring of embryonic radial glia (Aimone et al., 2014). In the future, a fully unbiased approach will reveal the

relations between these important cell types in full molecular detail.

These studies are some of the first steps along a road that will soon become well-trodden, and deservedly so. Single-cell analysis has come a long way in the last few years, and the technical advances have been astounding. Both studies in this issue used markers to select cells for study. As single-cell gene expression analysis gets cheaper, faster, and more accessible, we will likely see more and more examples where this power tool is used directly: instead of starting with a marker-based population, the entire tissue can be analyzed as large numbers of single cells. Cells can then be identified not just by handfuls of markers, but by their entire gene expression profiles, and the risk of misinterpretation due to the lack of specificity of markers can be avoided. Together, the studies showcase the power of single-cell analysis to elucidate the heterogeneity of complex cell populations. In particular, they show that a combination of advanced experimental and computational methods can be used to dissect developmental lineages in the neurogenic regions of the brain. More generally, they illustrate how rapidly biology is turning into a quantitative science of big datasets, powerful algorithms, and sophisticated analysis.

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