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A Time and Place for Understanding Neural Stem Cell Specification

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http://dx.doi.org/10.1016/j.devcel.2014.06.023

The regulation of neural stem cells is key to their use for repair. Reporting in this issue of *Developmental Cell*, Dirian et al. (2014) identify an adult neural stem cell population surprisingly distinct in Notch independence, lack of radial glia hallmarks, and late contribution to neurogenesis in a strikingly region-specific manner.

Organs differ profoundly in their rates of cell addition and turnover, and the brain has been seen for the longest time as an organ with little to no turnover. However, some vertebrate brains, such as that of zebrafish, continue to grow in adulthood; even in mammalian brains, some regions generate thousands of new neurons daily (Grandel and Brand, 2013). Importantly, however, the continuation of neurogenesis is highly region specific and species specific, with different brain regions in different species continuing neurogenesis (Grandel and Brand, 2013). Thus, key questions in the field are related to the regulation of region-specific continuation of neurogenesis and the developmental mechanisms that determine whether cells continue to generate neurons throughout the organism's lifetime or instead stop at some point. To answer these crucial issues, it is essential to understand the origin of long-term neural stem cells (NSCs) and identify the mechanisms regulating their behavior from development into adulthood—an important task that Dirian et al. (2014) has solved in this issue of *Developmental Cell*.

Dirian and colleagues (2014) addressed these key issues in the zebrafish forebrain, using the dorsal division (pallium) as a model system. In the zebrafish, as well as in different mammalian species, NSCs typically divide fast in development but are largely quiescent in the adult (Doetsch et al., 1999; Adolf et al., 2006). It is therefore a key question whether some of these fast proliferating and actively neurogenic cells in the developing brain indeed have the capacity to contribute to the more laid-back adult NSCs (aNSCs). Dirian and colleagues (2014) answered this by genetic fate mapping the fast dividing and active neurogenic cell population using her4 (a Notch target transcription factor)-driven inducible forms of the Cre recombinase at different developmental stages. They observe that even when they label these cells at very early stages (2 days postfertilization [dpf]), the progeny of these cells continue as NSCs in neurogenesis 3 months later. The surprise came when Dirian and colleagues (2014) found this to be the case only in one region (the dorsomedial portion) of the pallium, whereas the lateral portion of the pallium clearly originated from an unlabeled pool of NSCs. So where do the NSCs of the lateral pallium, which also continues to undergo neurogenesis into adulthood, originate?

Given the strict region-specific organization of the brain early during development and the evidence from mouse showing that aNSCs maintain their regional identity at least from early postnatal stages (Merkle et al., 2007), the authors reasoned that the cells in the lateral pallium could come from a particular domain lateral to the roof of the neural plate, a region equivalent in the embryo to where these NSC clones were found in the adult. In order to shed light on this, they elegantly used focal uncaging of caged cyclofen by laser light at 1.5 dpf at various positions and directly demonstrated that cells close to the roof plate give rise to cells in the lateral pallium, including radial glial-like aNSCs. Strikingly,

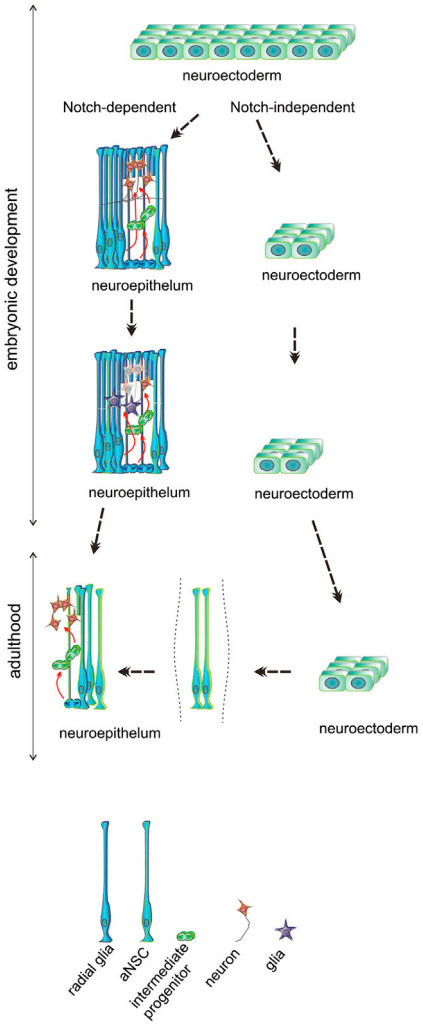

Figure 1. Model Depicting the Developmental Origin of Two Coexisting Populations of Adult Neural Stem Cells

Neuroepithelial cells, the earliest type of neural stem cells emerging in development (top), go on to diverge in a region-specific manner into Notch signaling-dependent radial glial cells undergoing neurogenesis (left), as observed in the dorsomedial domain of the telencephalon in Dirian et al. (2014), and the persisting neuroepithelial cells, restrained from neurogenesis and remaining in a Notch-independent state (right), as observed in the lateral pallium by Dirian et al. (2014). These more immature neural stem cells also then proceed to generate neurogenic radial glial cells that also act later as adult neural stem cells and persist as a small reserve stem cell pool into adulthood.

only a few cells during development give rise to the rather big domain of the lateral pallium, thus going from dwarf to giant. Indeed, clones, the progeny of a single cell, are up to 40 times larger in the lateral pallium than in the dorsomedial pallium, where NSCs also self-renew but generate many fewer progeny.

Taken together, the authors uncovered two distinct, spatially separated ancestors for aNSCs: her4-positive cells in the germinal zone of the pallium generate dorsomedial aNSCs, whereas the progenitors adjacent to the telencephalic roof plate generate the aNSCs of the lateral pallium. These progenitor pools remain segregated during the entire developmental process of the brain and are strikingly asynchronous in undergoing neurogenesis. The her4-negative ancestors of the lateral pallium do not undergo neurogenesis until later, whereas their her4+ neighbors do so from 2 dpf on.

So what are these her4-negative cells? Her4+ cells undergo neurogenesis, have a radial glia identity, and are Notch dependent, as expected from their her4 expression, a typical Notch target. In contrast, the ancestors of the lateral pallium NSCs close to the roof plate cells do not express her4 and do not generate neurons at early stages, are Notch independent, and resemble neuroepithelial cells lacking glial markers (Figure 1). This distinction in these NSC characteristics is of profound importance, as work led by Laure Bally-Cuif has previously uncovered such domains of cells that are held back from active neurogenesis in Notch-independent signaling centers present in the early zebrafish neural plate (Geling et al., 2004; Ninkovic et al., 2005). Interestingly, these centers are often located at boundaries and margins of a tissue. For example, a set of undifferentiated NSCs also resembling neuroepithelial cells (NEC) are located at the ciliary marginal zone in the retina and midbrain in regions that undergo continued growth (Brand and Livesey, 2011; Devès and Bourrat, 2012). Intriguingly, the marginal zone NSCs are set aside very early in development and do not acquire radial glia markers but rather retain neuroepithelial hallmarks (see also Grandel and Brand, 2013). Most strikingly, Dirian et al. (2014) demonstrate that the two populations of NSCs (her4− NECs; her4+ RGCs) are not only present in development, but this dichotomy also persists into adulthood. Indeed, the small persisting her4-negative, Notch-independent NSC pool seems to be responsible for the constant replenishment of the aNSCs in the lateral pallium in a Notch-independent manner. Thus, this work identifies for the first time disparate sets of aNSCs that follow a rather distinct developmental logic and persist at a different maturation state into adulthood.

One key implication of this is that the more "immature" her4-negative, Notch-independent NSCs may be an undifferentiated reserve pool for the case of emergency. Indeed, the zebrafish brain is a role model for neural regeneration, and these newly discovered immature NSCs may be key to achieving regeneration when the other (Notch-dependent) NSCs (Chapouton et al., 2010) have been wiped out. Dirian et al. (2014) examined this by eliminating the dorsomedial pool of NSCs by Notch inhibition at 15 dpf. Although the Notch-independent NSCs can transverse into her4+ NSCs and replenish neurogenesis in the lateral pallium, they cannot replenish her4+ NSCs in the dorsomedial pallium, indicative of profound differences between these regions and the very distinct pools of NSCs they contain. This highlights the need to activate NSCs with the appropriate regional identity and developmental origin for repair. Thus, the journey to regenerate neurons after brain injury starts at development, and it is apparent that zebrafish is not only one of the champions in regenerating the CNS, but also a champion for elucidating basic principles of neurogenesis from development to adulthood.

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