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Identifying the Unique Role of Notch3 in Adult Neural Stem Cell Maintenance

Joshua D. Rieskamp,[1,2] Jiyeon K. Denninger,[2] and Tyler J. Dause[2]

[1]Department of Neuroscience, Wexner Medical Center and [2]Department of Psychology, Ohio State University, Columbus, Ohio 43210

Review of Kawai et al.

The adult brain of most mammalian species retains distinct areas of neurogenesis that potentially contribute to the proper functioning of neural circuits. One of these niches, the subependymal zone (SEZ), also commonly referred to as subventricular zone, harbors neural stem cells (NSCs) that, in rodents, generate new neurons that migrate to the olfactory bulb under physiological conditions (Kriegstein and Alvarez-Buylla, 2009; Gage and Temple, 2013). To optimally contribute to the function of neural circuits, NSCs must be able to sense signals arising locally within the SEZ niche or from distant brain regions and respond by adjusting the balance between proliferation and quiescence. Quiescent NSCs lay dormant, but poised for cell division, until transitioning to activated NSCs, which generate transit-amplifying progenitors. Transient-amplifying progenitors undergo several rounds of proliferation before differentiating into neuroblasts, which, in turn, become immature neurons (Doetsch et al., 1999). Maintenance of stem cell pools and therefore normal neural function depends on the proper control of the processes maintaining quiescence or initiating activation of NSCs.

The Notch pathway has been extensively studied in the regulation of both embryonic and adult stem cells (Chiba, 2006). Mammals have four Notch receptors (Notch1–4), which can interact with five possible ligands (Delta1, Delta3, Delta4, Jagged1, and Jagged2). Receptor–ligand binding leads to cleavage of the receptor and release of the Notch Intracellular Domain. Canonically, Notch Intracellular Domain then translocates to the nucleus to form a transcriptional complex with Recombination Signal-Binding Protein For Immunoglobulin Kappa J Region and Mastermind-Like Transcriptional Coactivator to modulate transcription of target genes, such as the well-studied hairy and enhancer of split-related gene family (Ables et al., 2011). Two critically important functions of the Notch family are the maintenance of stem cell populations and correct lineage commitment during differentiation in several organs, including the brain (Stier et al., 2002, Kumano et al., 2003; Fre et al., 2005; van Es et al., 2005; Andersson et al., 2011). Prior work has shown that inactivation or deletion of Notch1 or Recombination Signal-Binding Protein For Immunoglobulin Kappa J Region leads to altered stem cell proliferation and abnormal neurogenesis (Hitoshi et al., 2002; Mizutani et al., 2007; Taylor et al., 2007; Imayoshi et al., 2010, Basak et al., 2012). In a recent article in *The Journal of Neuroscience*, Kawai et al. (2017) demonstrated that Notch3 also regulates NSCs in the SEZ.

Using *in situ* hybridization, Kawai et al. (2017) found that Notch3 is preferentially expressed within the lateral and ventral domains of the SEZ. FACS, using selective markers to isolate NSCs from the SEZ, revealed more abundant Notch3 mRNA in quiescent NSCs relative to activated NSCs, whereas the opposite was true of Notch1 mRNA. Furthermore, global Notch3 knockout (Kitamoto et al., 2005) significantly reduced quiescent NSCs, transient-amplifying progenitors, and neuroblasts, but did not alter the number of activated NSCs. This suggests that Notch3 deletion increased NSC transition from quiescence but prevented complete lineage progression. In addition to global Notch3 deletion, which could impact development, knockdown of Notch3 in the adult SEZ via a lentiviral shRNA expression vector increased NSC proliferation, indicating that acute Notch3 reduction in adulthood is sufficient to increase NSC activation. Together, these results suggest that Notch3 functions to inhibit the activation of quiescent NSCs in the SEZ and also may be required for normal lineage progression of activated NSCs.

Expanding on these findings, Kawai et al. (2017) treated Notch3-null mice with the antimitotic drug AraC, which eliminates rapidly dividing cells, such as activated

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Correspondence should be addressed to Joshua D. Rieskamp, 1835 Neil Avenue, Psychology Building, Columbus, OH 43210. E-mail: rieskamp.6@osu.edu.

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NSCs, transient-amplifying progenitors, and neuroblasts but spares quiescent NSCs, which can repopulate the SEZ progenitor pool after AraC is removed. Interestingly, Notch3 knock-out mice did not differ from controls in the proportion of quiescent NSCs labeled by a 1 h pulse of the mitotic marker EdU following AraC withdrawal, suggesting that, in this context, Notch3 did not change the ability of quiescent NSCs to transition to an activated state. This finding contrasts with the results of the acute Notch3 knockdown by shRNA, which increased NSC proliferation. The authors attribute the conflicting results to a possible difference in Notch3 function in homeostatic versus regenerative conditions. While it would be interesting to investigate the importance of Notch3 for maintaining neurogenesis in regenerative conditions, the authors did not assess whether Notch3 deletion changed the size of the recovering progenitor pool after AraC withdrawal.

The work of Kawai et al. (2017) advances our knowledge of NSC maintenance through the key finding that Notch signaling exerts distinct effects on the self-renewal of SEZ NSCs depending on which Notch receptor types are active in a given NSC (Fig. 1). Notch receptor expression aligned well with NSC developmental stage: Notch3 was robustly expressed in quiescent NSCs, whereas Notch1 was more likely to be expressed in activated NSCs and transient-amplifying progenitors. In accordance with this expression pattern, Notch3 deficiency selectively depleted quiescent NSCs while leaving activated NSC number unchanged, supporting a role for Notch3 in maintaining the quiescent NSC pool. These findings complement previous work showing that Notch1 deficiency reduced activated NSCs, transient-amplifying progenitors, and neuroblasts without depleting quiescent NSCs, which suggests that Notch1 acts primarily at the level of activated NSC proliferation (Basak et al., 2012). Furthermore, the distinct functions of the two Notch receptors align well with findings in zebrafish (Alunni et al., 2013), suggesting that similar regulatory mechanisms for NSC maintenance are shared across evolution and thus may be particularly indispensable for the functional contribution of NSCs to the adult nervous system.

Although the distinct functions of the receptors on NSC maintenance might be attributed in part to their differential expression patterns, some degree of overlap in expression is expected, as approximately one-third of quiescent NSCs expressed Notch1 in the current study. Therefore, it is worth considering additional mechanisms that might contribute to Notch receptor functional diversity. For example, the receptors could preferentially activate unique intracellular effectors or transcriptional targets (Andersson et al., 2011). In addition, different degrees of cross talk with other cell-extrinsic signaling pathways might distinguish the effects of the two Notch receptors (Aguirre et al., 2010). Finally, receptor–ligand interactions may differ between Notch3 and Notch1 due to post-translational modification of the receptors or differences in the Notch ligand supplied by neighboring cell populations. The latter two possibilities seem likely because, when Kawai et al. (2017) overexpressed Notch3 and Notch1 intracellular domains *in vitro*, in the ab-

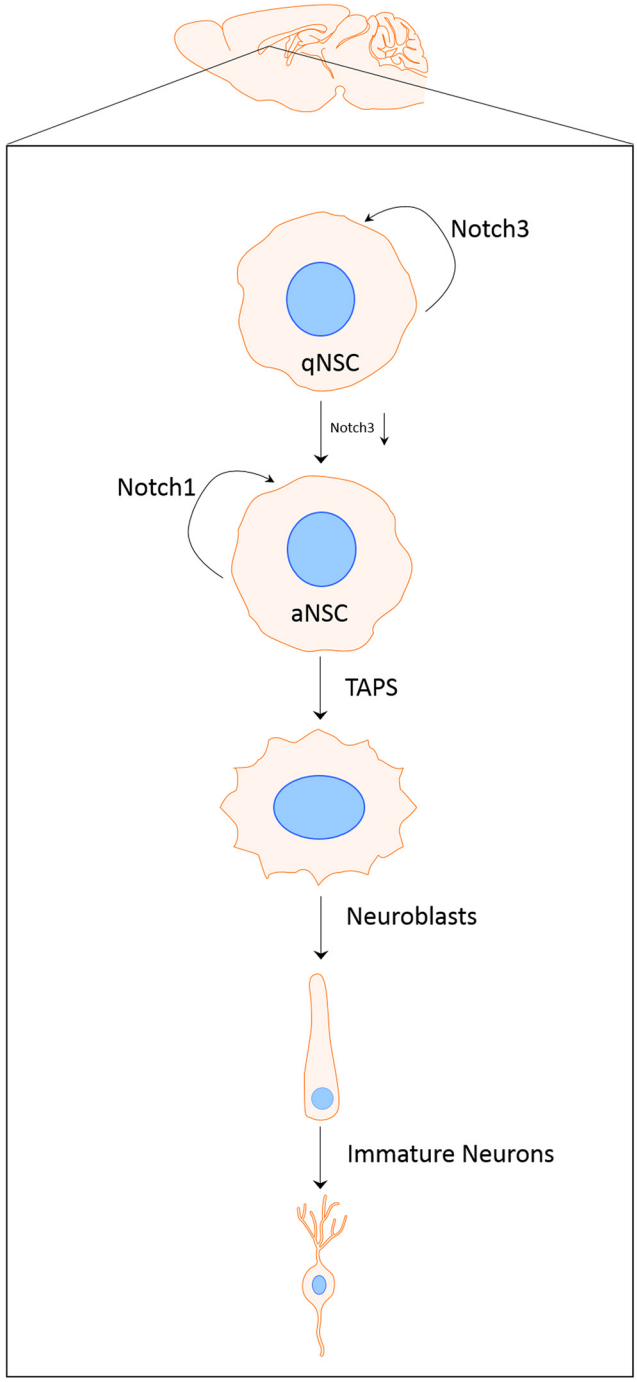

Figure 1. Notch signaling regulates the activation of NSCs in the SEZ. Notch3 maintains the pool of quiescent NSCs (qNSC) within the lateral domain of the SEZ. Conversely, Notch1 promotes proliferation of activated NSCs (aNSC), which then differentiate into transient-amplifying progenitors (TAPS), neuroblasts, and immature neurons that migrate to and populate the olfactory bulb.

sence of SEZ niche-derived cell-extrinsic input, the two domains produced similar inhibitory effects on NSC proliferation, despite the distinct effects of the receptors *in vivo*.

A second key finding of Kawai et al. (2017) is that, in the SEZ, Notch receptor expression differs not only by developmental stage, but also anatomical region, revealing an interaction with the positional identity of SEZ NSCs. The most salient consequence of positional identity is the tight coupling between spatial location of an NSC in the lateral ventricle and the type of olfactory bulb interneuron it produces (Lim and Alvarez-Buylla, 2016). In the study by Kawai et al. (2017), Notch3 expression exhibited a spatially restricted pattern, and Notch3 insufficiency impaired production of olfactory bulb Calbindin-expressing periglomerular interneurons derived from the lateral and ventral domains of the SEZ, where Notch3 expression is normally high. Conversely, production of tyrosine hydroxylase-positive interneurons derived from the dorsal SEZ, where Notch3 expression is normally low, was not impaired. Surprisingly, however, calretinin-positive neurons were reduced in number, even though these neurons are preferentially derived from the medial SEZ where Notch3 expression is normally low and quiescent NSC number was not reduced. This might be attributable to compensatory mechanisms in response to constitutive Notch3 deficiency. Regardless, the low level of Notch3 expression and lack of impaired neurogenesis in the dorsal SEZ imply that Notch3 in this region is not necessary for maintenance of the stem cell population as it is in other regions of the SEZ. While Kawai et al. (2017) did not explore spatial expression patterns of Notch1 and Notch2, another Notch paralog shown to be expressed in the SEZ (Basak et al., 2012), it is tempting to speculate that these Notch receptors also exhibit spatially restricted expression which could explain the reduced reliance of the dorsal SEZ on Notch3. If so, the regulation of different regional pools of SEZ NSCs by distinct Notch receptors might be an important mechanism for fine-tuning the relative quantities of olfactory bulb neuronal subtypes produced.

A central question facing the field of adult neurogenesis is how the pool of NSCs residing in the adult mammalian neurogenic niches maintains the lifelong balance between self-renewal and production of new neurons and glia. This question has important implications for understanding how NSC populations contribute to brain physiology under both healthy and pathological conditions that place differing demands on self-renewal and favor production of particular neuronal or glial lineages. While many questions remain, the work of Kawai et al. (2017) identifying a unique contribution of Notch3 to the regulation of specific SEZ NSC subpopulations is an important advance. It encourages continued research into the functional diversity and regional expression of signaling pathway components in neurogenic niches. Such efforts will continue to yield important insights into how endogenous NSC populations are maintained and to inform strategies to optimize stem-cell-based therapies.

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