COMMEMORATIVE LECTURE

Molecular and Cellular Mechanisms Contributing to the Regulation, Proliferation and Differentiation of Neural Stem Cells in the Adult Dentate Gyrus

Fred H. Gage

Laboratory of Genetics, Salk Institute of Biological Studies, California, USA

(Received for publication on January 16, 2010)

Adult neural stem cells (NSCs) are a potential endogenous source for neuronal cell replacement in the diseased adult central nervous system (CNS). However, the recruitment of adult NSCs for repair is hampered by the current lack of knowledge about the cellular and molecular mechanisms that control their behavior *in vivo*. We have previously demonstrated that environment-derived signals control the fate choice of adult NSCs. More recently we have provided evidence that in the adult hippocampal dentate gyrus – one of the two neurogenic regions of the adult CNS – specialized astrocytes provide signals that instruct NSCs to adopt a neuronal fate. In this review I will examine *in vitro* and *in vivo* the molecular mechanisms underlying the neuronal fate instruction of adult NSCs by the local astrocyte population in the adult hippocampus. In particular, I will focus on the Wnt family of proteins, which we have found to be expressed in adult hippocampal astrocytes; our preliminary studies have also shown that these proteins enhance the generation of neurons from adult NSCs *in vitro*. (Keio J Med 59 (3) : 79–83, September 2010)

***Keywords*: neurogenesis, hippocampus, proliferation, differentiation, survival**

Introduction

In the neurogenic niche of the adult mammalian brain, self-renewing neural stem cells (NSCs) give rise to committed neuronal progenitors within the subgranular zone (SGZ) of the dentate gyrus (DG).[1] Astrocytes are an essential cell population that defines the SGZ niche, and astrocyte-derived factors have instructive effects that promote adult neurogenesis.[2,3] Recently, we showed that Wnt3 expression persists in the adult hippocampus and that Wnt3 is released by astrocytes to regulate adult neurogenesis *in vitro* and *in vivo*.[4] In the canonical Wnt/β-catenin pathway, TCF transcription factors transduce Wnt/β-catenin signals to activate downstream target genes.[4–9] However, the target gene(s) of Wnt/β-catenin signaling responsible for promoting adult neurogenesis have not been identified. Moreover, the regulatory mechanism underlying Wnt-mediated neuronal differentiation has not yet been elucidated.

NeuroD1 is a proneural bHLH transcription factor essential for the development of the central nervous system (CNS), in particular the generation of granule cells in the hippocampus and cerebellum.[10,11] Environmental signals regulate adult neurogenesis at least in part through the activation of *NeuroD1*.[12,13] In preliminary studies, we showed that overexpression of NeuroD1 was sufficient to promote neuronal differentiation in adult hippocampal neural progenitors,[14] whereas deletion of *NeuroD1* resulted in decreased survival and maturation of newborn neurons.[15] Thus, we hypothesized that astrocyte-derived Wnt signals might directly or indirectly regulate the transcription of *NeuroD1* to control the transition of NSCs to committed neuronal progenitors.

The HMG-box transcription factor Sox2 is expressed in embryonic stem cells and most uncommitted cells in the developing CNS.[16–18] Sox2, which can be detected

---

Dr. Gage is the recipient of the 2008 Keio Medical Science Prize.

Presented at the Keio Medical Science Prize Commemorative Symposium, November 22, 2008.

Reprint requests to: Fred H. Gage, PhD, Laboratory of Genetics, Salk Institute of Biological Studies, 10010 N. Torrey Pines Rd., La Jolla, CA 92037, USA, E-mail: gage@salk.edu

in cells of the mouse blastocyst, maintains precursor cells in a multipotent state.[19–21] During CNS development, Sox2 prevents neurogenesis, and forced expression of Sox2 results in the loss of proneural cells.[21,22] Whereas overexpression of *Sox2* in neural progenitor cells derived from embryonic ventricular zone permitted the differentiation of progenitors into astroglia, it inhibited neurogenesis.[23] Although these analyses indicate that Sox2 is a transcriptional repressor of neuronal target genes during development, the exact nature of Sox2 regulation during adult neurogenesis remains elusive.

Here I summarize recent evidence that the transcriptional activation of *NeuroD1* is dependent on canonical Wnt/β-catenin activation and removal of Sox2 repression from the *NeuroD1* promoter in a sequence-specific manner. These studies provide evidence for possible crosstalk between Sox2 and Wnt/β-catenin signaling, which represents an important mechanism underlying adult granule cell neuronal differentiation through NeuroD1.

Characterization of the Effects of Astrocyte-derived Wnt3 on the Neuronal Differentiation of Adult Neural Stem Cells

To test the hypothesis that Wnt signaling is involved in the regulation of neuronal differentiation of adult hippocampal progenitors (AHPs) induced by hippocampal astrocytes, GFP-expressing AHPs were co-cultured for 4 days with hippocampal astrocytes in the presence or absence of secreted Frizzled-related proteins (sFRPs), which are thought to inhibit Wnt signaling by competing with Frizzled receptors for Wnt ligand and by forming complexes with Frizzled. Differentiation into neurons was evaluated every 24 hours by quantifying the percentage of AHPs expressing doublecortin, a microtubule binding protein that is expressed by at least a subset of proliferating neuroblasts and immature postmitotic neurons in the adult dentate gyrus. In addition, we determined the percentage of AHPs that expressed the mature neuronal marker MAP2ab after 4 days of co-culture. At all time points, the percentage of AHPs that differentiated into neurons was significantly decreased in the presence of sFRP2/3, indicating that Wnts participate in the previously described induction of neuronal differentiation of AHPs by hippocampal astrocyte-derived factors.[4] Furthermore, we analyzed astrocyte factors through gene expression arrays of hippocampal astrocytes compared to other cells and revealed several relevant changes in expression of genes whose proteins could greatly affect neurogenesis.[3]

Characterization of the Wnt-signaling Pathway Regulating the Neuronal Differentiation of Adult Neural Stem Cells

The interaction of Wnts with their receptors can trigger several signaling pathways, including the "canonical" β-catenin pathway and the "non-canonical" β-catenin-independent pathways. The β-catenin pathway has been primarily linked to cell proliferation during development; more recently, however, it has also been shown to influence fate determination and differentiation in several different organ systems during development. To examine whether hippocampal astrocyte-derived Wnts stimulated β-catenin signaling in AHPs, the Wnt/β-catenin signaling reporter Super8XTOPflash was electroporated into AHPs that were subsequently cultured in the presence or absence of a hippocampal astrocyte feeder layer. Co-culture with hippocampal astrocytes resulted in reporter activation, suggesting that hippocampal astrocytes stimulate Wnt signaling within AHPs. Next, AHPs were electroporated with the Wnt/β-catenin signaling reporter TOPGAL and cultured on hippocampal astrocytes for 4 days. Antibodies to β-galactosidase were used to track the activation of Wnt/β-catenin signaling within individual cells. β-Galactosidase expression was never observed in the GFAP-positive astrocyte population, which comprises not only the hippocampal astrocyte feeder layer but also the small fraction of AHPs that differentiate into astrocytes. In contrast, we found expression of the reporter almost exclusively in cells (>95% of the β-galactosidase-positive cells; n=300) that were positive for the neuronal markers doublecortin or MAP2ab, indicating that Wnt/β-catenin signaling is associated with the population of AHPs that differentiated into neurons.[4]

Gain and Loss of Function of Wnt Signaling in the Hippocampal Neurogenic Niche

We tested whether Wnt signaling is involved in the regulation of neurogenesis *in vivo*. Adult BATGAL mice were injected with a single dose of BrdU and sacrificed 24 hours later. Wnt/β-catenin pathway activity was observed in BrdU-positive cells in the SGZ and in doublecortin-expressing cells with morphological features of proliferating neuroblasts. These *in vivo* results are consistent with our *in vitro* finding that Wnt/β-catenin signaling regulates neuronal fate commitment and neuroblast proliferation. To block Wnt signaling *in vivo*, we used a secreted mutant Wnt protein in which 71 amino acids had been deleted from the carboxy-terminus of murine *Wnt1* and had been shown to non-autonomously block *Wnt* signaling in different *in vivo* assays. The cDNA encoding for this dominant negative Wnt protein (*dnWnt*) was cloned into the recombinant lentivirus (LV) vector *CSC-PPT-CMV-IRES-GFP-WPRE*, which contains an expression cassette under the control of the

CMV promoter and an IRES-GFP cassette to allow the identification of infected cells. *LV-dnWnt* or control LV encoding for GFP was stereotactically injected into the DG of young adult rats (8-9 weeks; n=8). After a 3-week period to allow transgene expression and to minimize confounding factors caused by the surgery, animals were injected with 200 mg/kg body weight BrdU daily for 7 days to label proliferating AHPs and their progeny. Animals were perfused 24 hours after the final BrdU injection. Compared to LV-GFP-injected animals, the LV-dnWnt-injected animals showed a dramatic reduction in the number (11170 ± 1122/mm^3 and 2751 ± 281.4/mm^3, respectively, p<0.0001, Student's t-test) and in the percentage of BrdU-positive cells that differentiated into neurons (57.62 ± 4.65% and 31.03 ± 5.48%, respectively, p<0.005, Student's t-test), resulting in a total ~8-fold reduction in neurogenesis in LV-dnWnt-injected animals in the infected areas (i.e., number of BrdU-positive cells per DG volume multiplied by the percentage of BrdU-positive cells that differentiated into neurons; 6302 ± 571.7 new doublecortin-positive neurons/mm^3 vs. 813.6 ± 118.7 new doublecortin-positive neurons/mm^3).[4] We further showed that orphan nuclear receptor Tlx played an important role in survival and differentiation of adult neurons in the DG, potentially acting through Wnt/beta-catenin signaling.[24,25]

Induction of Neurogenesis of Adult Neural Stem Cells Through the Manipulation of Wnt Signaling *In Vivo*

We tested whether increased Wnt signaling is sufficient to enhance adult hippocampal neurogenesis. The cDNA encoding for Wnt3 and for GFP were cloned into the LV vector *CCL-PPT-PGK-IRES-GFP-WPRE*. The expression cassette of this vector is under the control of the *PGK* promoter, which allows higher levels of transgene expression than the CMV promoter. *LV-Wnt3* or *LV-GFP* was stereotactically injected into the DG of mature (15 weeks) adult rats (n=6). Within the infected areas, LV-Wnt3-injected animals showed a significantly increased percentage of BrdU-positive cells expressing doublecortin compared to LV-GFP-injected animals (53.93 ± 6.76% and 70.29 ± 4.29%, respectively, p<0.05, Mann-Whitney rank sum test). These newborn immature neurons were often found in large clusters of more than 8 DCX/BrdU double-labeled cells in *LV-Wnt3*-injected animals but not in control animals.[4] In addition we revealed that the Cdk5,[26] NMDA receptor[27,28] and Mash 1[29] regulated and controlled the induction of neurogenesis of adult neural stem cells *in vivo*.

Functional Consequence of Loss of Wnt Signaling in the Hippocampal Neurogenic Niche

Given the fundamental role of the hippocampus in processes underlying certain forms of learning and memory, it has been speculated that newborn granule cells contribute to cognition. However, previous strategies aiming to causally link newborn neurons with hippocampal function used ablation strategies that were not exclusive to the hippocampus or that were associated with substantial side effects, such as inflammation. We used a lentiviral approach to specifically block neurogenesis in the DG of adult male rats by inhibiting Wnt signaling, which is critically involved in the generation of newborn neurons, using a dominant-negative WNT (*dnWNT*). We found a level-dependent effect of adult neurogenesis on the long-term retention of spatial memory in the water maze task: rats with substantially reduced levels of newborn neurons showed less preference for the target zone in probe trials > 2 weeks after acquisition compared to control rats. Furthermore, animals with strongly reduced levels of neurogenesis were impaired in a hippocampus-dependent object recognition task. Social transmission of food preference, a behavioral test that also depends on hippocampal function, was not affected by knockdown of neurogenesis. We identified a role for newborn neurons in distinct aspects of hippocampal function that will lay the groundwork to further elucidate, using experimental and computational strategies, the mechanism by which newborn neurons contribute to behavior.[30]

Transition from Sox2-expressing NSCs to NeuroD1-positive Cells in Adult Dentate Gyrus

To visualize the transcription factors determining the transition from the undifferentiated state of NSC to neuronal differentiation, we stained adult hippocampus sections using several antibodies. We used the *Sox2* promoter-driven *EGFP* transgenic mouse[1] and compared the *EGFP* expression pattern with that of *NeuroD1*. *NeuroD1*-expressing cells were clearly detected in the DG area. Importantly, Sox2- and NeuroD1-positive cells were mutually exclusive, and neither Sox2 nor NeuroD1 was immunoreactive for astrocytic S100β. Cells expressing NeuroD1 were detected only in the inner layer of the SGZ of the DG, where adult neurogenesis continuously occurs.[12,31] Also, Sox2-positive cells were negative for the mature neuron marker NeuN, and NeuroD1-positive cells never co-localized with NeuN-positive cells.

To characterize the properties of NeuroD1-positive cells, we performed an immunohistochemical analysis with specific proneural markers. NeuroD1-positive cells co-localized with nestin, calretinin and doublecortin. Sox2-GFP cells colocalized with the stem cell marker nestin and only a few and weakly expressing GFP-positive cells colocalized with the weaker DCX.[1] A major proportion of the NeuroD1-positive cells co-localized with DCX, and a few of them were nestin-positive, suggesting that NeuroD1-positive cells had recently transitioned from Sox2-positive NSCs toward neurogenesis. NeuroD1-positive cells co-localized with Ki67, indicat-

ing that they are in the proliferating population in the DG. To examine the proliferative status of NeuroD1-expressing cells, BrdU (100 mg/kg) was injected once a day for 1 week into Fisher344 rats (7- to 8-week-old animals). Within the subgranular layer of the DG, NeuroD1-positive cells apparently incorporated BrdU. Taken together with an immunohistochemical analysis, it was confirmed that NeuroD1-expressing cells appeared in the mitotically active progenitor stage first, and they were mainly in immature neurons within the granule cell layer of the DG.

Based on the expression of NeuroD1 during adult neurogenesis and the essential role of NeuroD1 in newborn neurons in the adult hippocampus, we focused on the mechanism by which the transcription of the NeuroD1 gene is regulated to better understand neurogenesis in adult NSCs. To gain insight into the possible transcription regulation of NeuroD during the transition from NSCs to neuronal cells, we analyzed the DNA binding sequences of Sox2 and TCF/LEF transcription factors on the NeuroD1 and NeuroD2 promoters. The binding sites for the TCF/LEF and Sox2 transcription factors were found within 3 kb upstream of the promoter region of both the NeuroD1 and NeuroD2 promoters. Interestingly, some sequences overlapped. These overlapping DNA regulatory elements were conserved among the human, rat and murine NeuroD1 promoters. This observation led to the subsequent analysis of the functional significance of these overlapping sequences and their reciprocal control over neurogenesis in the adult DG.[32]

Does Wnt Signal through β-catenin to Activate TCF/LEF to Regulate Neurogenesis?

We now have evidence that NeuroD1 is up-regulated in early committed neuronal cells. To evaluate this effect *in vivo*, *LV-β-catenin shRNA* or control lentivirus (LV) expressing only *GFP* (*LV-GFP*) was stereotactically injected into the DG of young adult mice (i.e., 6 to 8 weeks old; n = 12/group). Two weeks after the injection, BrdU (100 mg/kg) was injected once a day for 1 week. Three weeks after the original injection, the animals were analyzed. Our results revealed that control LV-GFP injections resulted in many GFP-positive cells in the granule cell layer of the DG. Some of those GFP-positive cells were Sox2-positive and BrdU-positive, indicating that the lentivirus preferentially infected not only mature neurons but also proliferating cells, including undifferentiated NSCs.[32] This finding is consistent with our previous report in which LV-based tracing analysis of Sox2-positive cells within the DG was described.[1]

LV-β-catenin shRNA-infected cells were observed in the inner layer of the DG, and the GFP-positive cells expressing *β-catenin shRNA* (*shRNA-GFP*) also co-localized with Sox2. The shRNA-GFP cells co-localizing with Sox2 were labeled by BrdU, indicating that the cells in which the β-catenin gene was down-regulated survived and remained in an undifferentiated state.[32] Thus, compared to *dnWnt*, *LV-β-catenin shRNA* results in a partial loss of function; therefore, it is possible that small changes in Wnt signaling may regulate proliferation vs. differentiation, whereas complete loss of Wnt signaling would be more detrimental to the neurogenic niche. Thus, as with other molecules involved in these dynamic processes, there are gradients of activity, and the magnitude of signaling within a given signaling pathway may have qualitative as well as quantitative effects.

Discussion and Summary

Adult NSCs are a potential endogenous source for neuronal cell replacement in the diseased adult CNS. However, the recruitment of adult NSCs for repair is hampered by the current lack of knowledge about the cellular and molecular mechanisms that control their behavior *in vivo*. We have previously demonstrated that environment-derived signals control the fate choice of adult NSCs. More recently we have provided evidence that in the adult hippocampal DG – one of the two neurogenic regions of the adult CNS – specialized astrocytes provide signals that instruct NSCs to adopt a neuronal fate. Here I summarized the *in vitro* and *in vivo* molecular mechanisms underlying the neuronal fate instruction of adult NSCs by the local astrocyte population in the adult hippocampus. In particular, I focused on the Wnt family of proteins, which we have found to be expressed in adult hippocampal astrocytes; our studies have also shown that these proteins enhance the generation of neurons from adult NSCs *in vitro*.

Adult neurogenesis represents an extreme form of neural plasticity that has the potential to have significant impact on memory capabilities throughout life. It is clear from many reports that neurogenesis is regulated by many normal life conditions (e.g., stress, age and environmental enrichment), is impaired in a variety of neurological and psychiatric diseases, and can be actively regulated by both behavioral and pharmaceutical interventions (e.g., exercise, SSRIs and chemotherapy), suggesting that the birth of new neurons may be a mechanism to target when addressing neurological and psychiatric diseases. If neurogenesis is going to be used as a therapeutic target in addressing age- and stress-related disorders, for instance, it is important to understand the molecular and cellular mechanisms that control and regulate the process. We have uncovered a potential interaction between three separate molecular pathways that work together to sustain NSCs in the brain and regulate the generation of new neurons in the brain. Understanding the way these pathways coordinate to generate neurons will provide new targets to protect or enhance adult neurogenesis. A better understanding of how adult neurogene-

sis affects and contributes to the function of the hippocampus will help to address why the dysregulation of adult neurogenesis may relate to certain natural and neurological conditions.

Acknowledgments

I am particularly indebted to those scientists who collaborated with me on the projects reported in the review, including Tomoko Kuwabara, Sebastian Jessberger, Chichung Lie, Hong Jun Song, Hoonkyo Suh, Xinyu Zhao, Kinichi Nakashima, Ayumu Tashiro and Ahmet Denli. The work was supported by grants from NINDS/NIH and the McDonnell Foundation.

References

1. Suh H, Consiglio A, Ray J, Sawai T, D'Amour KA, Gage FH: *In vivo* fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. Cell Stem Cell. 2007; **1**: 515–528. PMCID: PMC2185820.
2. Song H, Stevens CF, Gage FH: Astroglia induce neurogenesis from adult neural stem cells. Nature. 2002; **417**: 39–44.
3. Barkho BZ, Song H, Aimone JB, Smrt RD, Kuwabara T, Nakashima K, Gage FH, Zhao X: Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. Stem Cells Dev. 2006; **15**: 407–421.
4. Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, Lein ES, Jessberger S, *et al*: Wnt signalling regulates adult hippocampal neurogenesis. Nature. 2005; **437**: 1370–1375.
5. Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R: Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. Development. 2000; **127**: 469–482.
6. Machon O, van den Bout CJ, Backman M, Kemler R, Krauss S: Role of beta-catenin in the developing cortical and hippocampal neuroepithelium. Neuroscience. 2003; **122**: 129–143.
7. Solberg N, Machon O, Krauss S: Effect of canonical Wnt inhibition in the neurogenic cortex, hippocampus, and premigratory dentate gyrus progenitor pool. Dev Dyn. 2008; **237**: 1799–1811.
8. Clevers H: Wnt/beta-catenin signaling in development and disease. Cell. 2006; **127**: 469–480.
9. Wexler EM, Geschwind DH, Palmer TD: Lithium regulates adult hippocampal progenitor development through canonical Wnt pathway activation. Mol Psychiatry. 2008; **13**: 285–292.
10. Liu M, Pleasure SJ, Collins AE, Noebels JL, Naya FJ, Tsai MJ, Lowenstein DH: Loss of BETA2/NeuroD leads to malformation of the dentate gyrus and epilepsy. Proc Natl Acad Sci USA. 2000; **97**: 865–870. PMCID: PMC15422.
11. Miyata T, Maeda T, Lee JE: NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus. Genes Dev. 1999; **13**:1647–1652. PMCID: PMC316850.
12. Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC: Excitation-neurogenesis coupling in adult neural stem/progenitor cells. Neuron. 2004; **42**: 535–552.
13. Tozuka Y, Fukuda S, Namba T, Seki T, Hisatsune T: GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. Neuron. 2005; **47**: 803–815.
14. Hsieh J, Nakashima K, Kuwabara T, Mejia E, Gage FH: Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. Proc Natl Acad Sci USA. 2004; **101**: 16659–16664. PMCID: PMC527137.
15. Gao Z, Ure K, Ables JL, Lagace DC, Nave KA, Goebbels S, Eisch AJ, Hsieh J: Neurod1 is essential for the survival and maturation of adult-born neurons. Nat Neurosci. 2009 Sep; **12**: 1090–1092.
16. Collignon J, Sockanathan S, Hacker A, *et al*: A comparison of the properties of Sox-3 with Sry and two related genes, Sox-1 and Sox-2. Development. 1996; **122**: 509–520.
17. Ferri AL, Cavallaro M, Braida D, *et al*: Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. Development. 2004; **131**: 3805–3819.
18. Nishimoto M, Fukushima A, Okuda A, Muramatsu M: The gene for the embryonic stem cell coactivator UTF1 carries a regulatory element which selectively interacts with a complex composed of Oct-3/4 and Sox-2. Mol Cell Biol. 1999; **19**: 5453–5465. PMCID: PMC84387.
19. Ambrosetti DC, Basilico C, Dailey L: Synergistic activation of the fibroblast growth factor 4 enhancer by Sox2 and Oct-3 depends on protein-protein interactions facilitated by a specific spatial arrangement of factor binding sites. Mol Cell Biol. 1997; **17**: 6321–6329. PMCID: PMC232483.
20. Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R: Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 2003; **17**: 126–140. PMCID: PMC195970.
21. Bylund M, Andersson E, Novitch BG, Muhr J: Vertebrate neurogenesis is counteracted by Sox1-3 activity. Nat Neurosci. 2003; **6**: 1162–1168.
22. Graham V, Khudyakov J, Ellis P, Pevny L: SOX2 functions to maintain neural progenitor identity. Neuron. 2003; **39**: 749–765.
23. Bani-Yaghoub M, Tremblay RG, Lei JX, *et al*: Role of Sox2 in the development of the mouse neocortex. Dev Biol. 2006; **295**: 52–66.
24. Zhang CL, Zou Y, He W, Gage FH, Evans RM: A role for adult TLX-positive neural stem cells in learning and behaviour. Nature. 2008; **451**: 1004–1007.
25. Qu Q, Sun G, Li W, *et al*: Orphan nuclear receptor TLX activates Wnt/beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. Nat Cell Biol. 2010 Jan; **12**: 31–40; sup 1–9.
26. Jessberger S, Aigner S, Clemenson GD, Jr., *et al*: Cdk5 regulates accurate maturation of newborn granule cells in the adult hippocampus. PLoS Biol. 2008; **6**: e272. PMCID: PMC2581629.
27. Tashiro A, Zhao C, Gage FH: Retrovirus-mediated single-cell gene knockout technique in adult newborn neurons *in vivo*. Nat Protoc. 2006; **1**: 3049–3055.
28. Tashiro A, Sandler VM, Toni N, Zhao C, Gage FH: NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. Nature. 2006; **442**: 929–933.
29. Jessberger S, Toni N, Clemenson GD, Jr., Ray J, Gage FH: Directed differentiation of hippocampal stem/progenitor cells in the adult brain. Nat Neurosci. 2008; **11**: 888–893.
30. Jessberger S, Clark RE, Broadbent NJ, Clemenson GD, Jr., Consiglio A, Lie DC, Squire LR, Gage FH: Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. Learn Mem. 2009; **16**: 147–154. PMCID: PMC2661246.
31. Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH: Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development. 2003; **130**: 391–399.
32. Kuwabara T, Hsieh J, Muotri A, *et al*: Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. Nat Neurosci. 2009; **12**: 1097–1105.