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For the Long Run: Maintaining Germinal Niches in the Adult Brain

Minireview

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The adult mammalian brain retains neural stem cells that continually generate new neurons within two restricted regions: the subventricular zone (SVZ) of the lateral ventricle and the dentate gyrus subgranular zone (SGZ) of the hippocampus. Though these cellular populations are spatially isolated and subserve different brain systems, common themes begin to define adult neurogenic niches: (1) astrocytes serve as both stem cell and niche cell, (2) a basal lamina and concomitant vasculogenesis may be essential components of the niche, and (3) "embryonic" molecular morphogens and signals persist in these niches and play critical roles for adult neurogenesis. The adult neurogenic niches can be viewed as "displaced" neuroepithelium, pockets of cells and local signals that preserve enough embryonic character to maintain neurogenesis for life.

Stem cell self-renewal and progenitor differentiation is regulated by the specialized microenviroment—or "niche"—in which these cells reside. Such niches are composed of soluble factors as well as membrane bound molecules and extracellular matrix (ECM). During brain development, most stem cells and their niches are spatially ephemeral and temporally transient as the cellular and molecular "puzzle" behind neurogenesis and morphogenesis is "assembled" and "disassembled" at a dazzling pace. In contrast, in the adult, neural stem cells and their niches are retained in restricted regions with their local developmental processes occurring for the life of the animal. It is now becoming clear that pieces of the embryonic developmental puzzle are retained for adult neurogenesis. Remarkably, classical developmental signals and morphogens like Notch, BMPs, Eph/ephrins, Noggin, and Shh seem to play important roles in maintaining adult neurogenic niches.

In the adult rodent brain, neurogenic stem cells are concentrated in the subventricular zone (SVZ) of the lateral ventricle wall and the dentate gyrus subgranular zone (SGZ) of the hippocampus (Figure 1). Throughout adult life, cells born in the SVZ traverse a long distance anteriorly through the rostral migratory stream into the olfactory bulb (OB), where they differentiate into interneurons. Neurons in the dentate gyrus are born locally in the underlying SGZ and migrate a short distance to integrate in the dentate gyrus (reviewed in Gage, 2000). Surprisingly, cells that have structural and molecular characteristics of astrocytes function as neurogenic stem cells in the SVZ and SGZ (see Figures 1 and 2; Doetsch et al., 1999; Imura et al., 2003; Laywell et al., 2000; Seri et al., 2001). The finding that SVZ and SGZ astrocytes are neurogenic stem cells challenges traditional views concerning brain development as well as stem cell identity. Studies in adult birds and developing mammalian cortex also demonstrate that radial glia, cells closely related to astrocytes, function as primary precursors for new neurons. Thus, cells classically considered to be in the astroglial lineage appear to serve as neurogenic stem cells.

Astrocytes outside of the SVZ and SGZ do not appear to be neurogenic in vivo under normal conditions. It is also not clear whether these cells can be induced to function as stem cells in adult animals. Interestingly, however, young astrocytes isolated from multiple brain regions before postnatal day 10 can give rise to neurospheres—neural stem cells in vitro (Laywell et al., 2000). The relationship and defining characteristics of germinal astrocytes versus parenchymal ("differentiated") astrocytes remains to be determined.

The neurogenic behavior of SVZ and SGZ progenitors appears determined by signals restricted to their niches. SVZ cells transplanted to another SVZ generate large numbers of neurons for the recipient animal OB, but SVZ cells transplanted to nonneurogenic brain regions are severely limited in their neurogenic potential (see Gage, 2000; Lim et al., 2000, and references therein). Cultured SGZ progenitors behave similarly, producing interneurons when grafted to SGZ but not to nonneurogenic targets. Interestingly, SGZ progenitors appropriately produce tyrosine hydroxylase-positive interneurons for the OB when grafted to the rostral migratory stream, between the SVZ and the olfactory bulb, suggesting that this microenvironment is instructive and not merely permissive (reviewed in Gage, 2000). It thus appears that the SVZ and SGZ stem cell microenvironments, in addition to maintaining the population of stem cells, also direct neuronal differentiation.

SVZ astrocytes (type B cells) are in intimate contact with all other SVZ cell types, including the rapidly dividing transit amplifying (type C cells) and the committed migratory neuroblasts (type A cells). The cell lineage is type B to C to A (Figure 1), with type B cells believed to be the self-renewing primary precursors. B and C cells cultured in serum-free medium in direct contact with monolayers of astrocytes proliferate to form colonies of young neurons (Lim and Alvarez-Buylla, 1999). Similarly, astroglial-derived soluble and membrane bound factors promote proliferation and neuronal fate for hippocampal stem cells (Song et al., 2002). Hence, astrocytes in these germinal layers, in addition to functioning as primary precursors for the new neurons, also participate in the creation of the microenvironment that stimulates neurogenesis.

In development and regenerative tissues, stem cell maintenance and differentiation is often contingent upon their proximity to a basal lamina (BL). The SVZ BL is rich in laminin and collagen-1, and there is an associated perivascular "connective" tissue comprised of fibroblasts and macrophages (Mercier et al., 2002). Mercier et al. propose that the BL concentrate and/or modulate cytokines/growth factors derived from local cells. The

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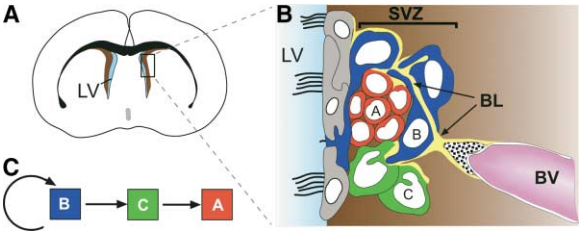

Figure 1. Adult SVZ Neurogenesis

(A) Coronal section through the adult mouse brain. Light blue shows the lateral ventricle (LV) space filled with cerebrospinal fluid. Boxed area is shown enlarged in (B).

(B) Architecture of the SVZ. B cells (dark blue) are the astrocytes that are the SVZ stem cell and also serve as niche cells (see text for details). Some of the B cells contact the ventricle lumen and have a single cilium (shown). C cells (green) are rapidly dividing, transit-amplifying cells derived from the B cells. C cells give rise to A cells (red), neuroblasts that migrate to the olfactory bulb, where they become local interneurons. A blood vessel (BV, pink) is shown with a perivascular macrophage (dotted fill); a basal lamina (BL, yellow) extends from the BV and interdigitates extensively with the SVZ cells. Ciliated ependymal cells (gray) line the ventricle walls and have been shown to produce Noggin, which is important for this niche. Noggin, BMPs, Shh, Notch, TGFα, Eph/ephrins, and VEGF play roles in regulation of this neurogenic niche (see text).

(C) SVZ lineage. C cells can behave as stem cells under the influence of excess EGF signaling (see text).

BL is extensively interdigitated with all SVZ cell types, and type B cells have the most extensive contact (Figure 1B). Perhaps this extensive attachment to the BL is important for type B cell maintenance of stem cell properties. Although the importance of BL contact is still hypothetical, it is interesting to recount the behavior of stem cells in embryonic brain development (Figure 3). Early neuroepithelial cells extend processes that contact the BL at the pial surface of the brain (Figure 3A). As the brain grows, radial glial contacts with the pial BL are maintained by radial process elongation (Figure 3B). Radial glia have been hypothesized to give rise to both

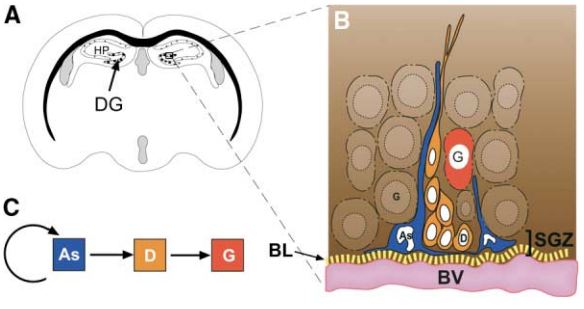

Figure 2. Adult SGZ Neurogenesis

(A) Coronal section through the adult mouse brain at the level of the hippocampus (HP). The dentate gyrus (DG, heavy dotted fill) is indicated by the arrow. The SGZ of the DG is shown enlarged in (B).

(B) Architecture of the SGZ. Astrocytes (As, dark blue) give rise to progenitors (D cells, orange), which mature into new granule cells (red G cells). These newly born granule cells integrate into the DG granule cell layer (brown G cells). Blood vessels (BV, pink) are found close to the SGZ layer, and we propose that a perivascular basal lamina (BL, yellow dotted line) exists here similar to that found in the SVZ. Shh and VEGF regulate the SGZ niche in vivo (see text).

(C) Lineage of the SGZ.

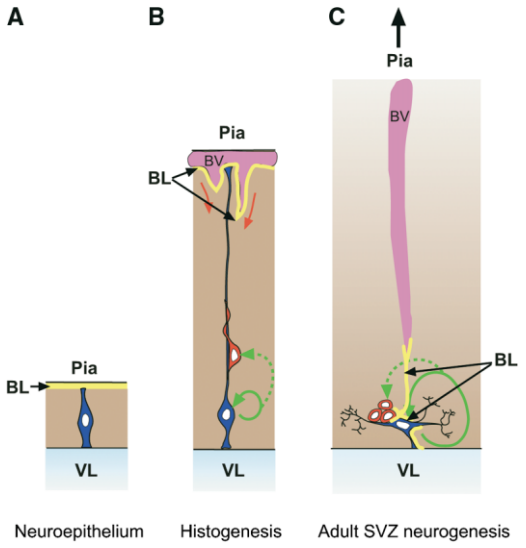

Figure 3. The Adult Neurogenic Stem Cell Niche: A Displaced Neuroepithelium?

Hypothetical relationship of stem cells from the neuroepithelial stage to the adult SVZ.

(A) Neural stem cells in the early neuroepithelium extend from the ventricle (bottom, light blue) to the basal lamina (BL, yellow) of the pial surface (top).

(B) As the brain grows during histogenesis, radial glial stem cells maintain contact with both the ventricle and pial BL by radial glial process elongation. These radial glial cells divide and likely self-renew (green solid arrow) as well as produce neurons (red), possibly through intermediates (green dotted arrow). The brain vasculature invades the parenchyma from the pial surface (pink vessel, red arrows), carrying with it the perivascular BL (yellow).

(C) Radial glial cells give rise to astrocytes later in development, and some come to reside in the adult SVZ. Here, some SVZ astrocytes contact the ventricle (see Figure 1B) and also maintain contact with a BL (yellow lines) carried in by local vasculature (pink). These astrocytes behave as stem cells in that they self-renew (green solid arrow) as well as produce neurons (red) through intermediates (dotted green arrow).

parenchymal, nonstem cell astrocytes as well as neurogenic astrocytes in the SVZ and SGZ. Perhaps SVZ/SGZ astrocyte stem cell behavior is in part dependent upon BL contact provided by the radial in-growth of blood vessels from the pial layer (Figures 3B and 3C). Studies assessing the requirement of the BL will be critical to our understanding of the adult stem cell niche.

There is a relationship between vasculogenesis and neurogenesis in the adult brain. Brain blood vessels in the SVZ are intimately associated with the BL (Mercier et al., 2002), and the BL is interdigitated with astrocytes that function as both stem cells and niche cells (Figure 1). In the SGZ, bursts of endothelial cell division are spatially and temporally related to clusters of neurogenesis (Palmer et al., 2000). Within these clusters of cell division, vascular endothelial growth factor (VEGF) receptors are seen. Intraventricular VEGF infusions increase proliferation of both SVZ and SGZ neuronal precursors; in the SGZ, VEGFR2 colocalizes with the immature neuronal marker doublecortin (Dcx), suggesting a direct action of VEGF on neuronal progenitors (Jin et al., 2002).

The carbohydrate moiety LeX, also known as SSEA-1 or CD15, is expressed in blood vessels and the surface

of the brain, regions rich in extracellular matrix and where a BL is present. Interestingly, LeX is also expressed in the SVZ and SGL where it appears to be associated with a subpopulation of astrocytes in vivo. Importantly, most SVZ cells that grow as neurosphere stem cells in vitro also express LeX (Capela and Temple, 2002). LeX has also been found on embryonic stem cells, where it is thought to influence blastocyst adhesion. Little is known about other carbohydrate moieties on neurogenic regions in the adult, but carbohydrate ectodomains on proteoglycans and other glycoconjugates like LeX are likely very important components of the adult neurogenic niche, probably involved in the presentation of growth factors on the surface of neural stem cells and their progeny and modulating their adhesion.

Another set of molecules associated with astrocytes in the neurogenic regions is the Eph/ephrin family of signaling molecules (Conover et al., 2000). SVZ astrocytes cells express ephrin B2/3 ligands, and intraventricular infusion of EphB2 ectodomain (which activates ephrin B2 on the astrocytes) increases astrocyte cell numbers, also inducing these cells to contact the ventricle. The increased proliferation results in the formation of polypoid hyperplasias that protrude into the ventricle lumen. Importantly, Eph/ephrins have also been shown to be critical for vasculogenesis, once again providing a possible molecular link between these two germinal compartments.

The Notch family of receptors participates in many developmental cell fate decisions and in certain contexts promotes an undifferentiated, precursor cell state. Notch1 and a cognate ligand, Jagged, are expressed in both the SVZ and SGZ (Stump et al., 2002). Retroviral induction of activated Notch at E9.5 promotes radial glial identity and produces dense clusters of SVZ astrocytes postnatally (Gaiano et al., 2000). Notch may participate in suppressing neuronal differentiation and maintaining precursor cell properties. In support of this notion, activated Notch in postnatal SVZ cells prevents migration to the olfactory bulb, suppresses neuronal differentiation, and decreases proliferation, creating a more "quiescent" cell type (Chambers et al., 2001). If Notch ligands (e.g., Jagged) are expressed later in the neurogenic lineage, a potential mechanism for feedback regulation of the niche emerges: accumulation of neuroblasts expressing Notch ligands activate Notch signaling in the stem cells, thereby suppressing neurogenesis. This could be a niche mechanism for feedback regulation of neuronal differentiation of stem cells or homeostasis. Notch signaling is context dependent, and it remains to be determined if it promotes terminal glial differentiation (Tanigaki et al., 2001) or a precursor state in adult germinal regions.

There have been many studies of the effect of EGF and FGF on SVZ cells, both in vitro and in vivo. Infusion of EGF or FGF into the brain ventricles dramatically expands the SVZ cell population (reviewed in Gage, 2000, and referenced in Tropepe et al., 1997). It was originally suggested that EGF amplifies the relatively quiescent neural stem cell in the SVZ, but recent work suggest that EGF primarily acts on the transiently amplifying populations (Doetsch et al., 2002). EGF induces C cells to divide and invade adjacent brain, and this expanded population of C cells displays multipotent stem cell properties in vitro. Little is known, however, of the normal endogenous role of EGF and FGF signaling in the control of progenitor proliferation. While EGF itself is not found in the SVZ, TGFα (which also activates the EGF receptor) is expressed, and TGFα knockouts have decreased SVZ proliferation and fewer new neurons reaching the olfactory bulb (Tropepe et al., 1997). One may wonder if this phenotype is due to depletion of neural stem cells or to a loss of a mitogenic signal for transit-amplifying cells. The finding that EGF-stimulated C cells can behave as stem cells in vitro (Doetsch et al., 2002) suggests that "stemness" may be more related to competence of a group of early precursors within a lineage rather than to a specific type of cell. This notion is also supported by reports indicating that early oligodendrocyte precursors can be induced to behave as stem cells in vitro (Kondo and Raff, 2000; Nunes et al., 2003). Hence, in the brain, the microenvironment may play a critical role in inducing stem cell competence or limiting the differentiation potential of early progenitors.

Sonic hedgehog (Shh) is an important morphogen in development and has been shown to regulate both SVZ and SGZ neural stem cells. Overexpression of Shh near the dentate gyrus increases proliferation and neurogenesis of SGZ cells; additionally, in culture, Shh maintains proliferation of adult hippocampal neuronal progenitors (Lai et al., 2003). Recently, Smoothened (*Smo*), the coreceptor for SHH, has been conditionally removed from neural precursors from E12.5 embryos by crossing floxed *Smo* ($Smo^{n/c}$) with Nestin-Cre (N^{cre}) animals (Machold et al., 2003); $Smo^{n/c};N^{cre}$ animals have less SVZ and SGZ cell proliferation, increased germinal zone apoptosis, and fewer neurons in their respective neurogenic targets. Notably, the mature brains of $Smo^{n/c};N^{cre}$ appear relatively normal, suggesting that Shh signaling in neuronal precursors after E12.5 is primarily important for postnatal and adult neurogenesis. Combining these genetic data with in vitro studies demonstrating fewer neural stem cells from these $Smo^{n/c};N^{cre}$ animals, the authors propose that Shh is a component of the neural stem cell niche, "maintaining" the stem cell population. It is not clear, however, if the reduced neurogenesis in these mice is due to a postnatal effect or an embryonic change in nestin-expressing precursors that is later manifested in the functioning of the SVZ. While the notion of Shh as a stem cell "maintenance" factor is intriguing, another explanation is that Shh may promote proliferation or support survival of the transit-amplifying (type C) cell in the SVZ. It will be very interesting to determine which cells in the SVZ and SGZ are expanded with increased Shh signaling as well as the populations that are depleted by the absence of Shh signaling.

Another group of early neural morphogens, the bone morphogenetic proteins (BMPs), also play an important role in adult brain germinal niches. In contrast to Shh, BMPs are considered dorsal morphogens. Interestingly, in the adult SVZ, both BMP and Shh signaling are intermixed within the same region. BMP signaling during development promotes astrocyte differentiation of SVZ precursors at the expense of oligodendrogliogenesis and neurogenesis. Adult SVZ cells themselves produce BMPs and their receptors. Noggin, a secreted BMP antagonist, is strongly expressed in ependymal cells. This locally derived BMP antagonist is thought to contribute

to the neurogenic niche for SVZ stem cells as it promotes neurogenesis both in vitro and in ectopic locations in vivo (Lim et al., 2000).

Recent work indicates that BMPs act in combination with leukemia inhibitory factor (LIF) to sustain self-renewal and suppress differentiation in embryonic stem cells. This inhibition is mediated through Id genes, which encode a major family of negative bHLH factor (Ying et al., 2003). Similarly, BMPs induce Id genes in neuroepithelial cells (Nakashima et al., 2001). The effects of BMPs in the SVZ/SGZ may also be mediated by Id genes and participate with Notch signaling—which has also been shown to activate Id genes (Reynaud-Deonauth et al., 2002)—to promote a stem cell state. In line with this notion is the fact that BMPs and Notch signaling decrease proliferation and induce an "astrocyte" phenotype in SVZ cells. Are BMP/Notch-induced "astrocytes" actually quiescent stem cells?

Thus, major developmental signaling pathways including Notch, Eph/ephrins, Shh, and BMPs are retained in adult germinal niches, where they appear to regulate important aspects of proliferation and differentiation. The precise cell types in the SGZ and SVZ where these pathways operate, the physiological conditions that trigger signaling in each pathway, and—perhaps most challenging—the interrelationship between them to produce the appropriate response are central questions for future research. We may draw some inspiration from the regulation of stem cells in other tissues like skin and blood where remarkably similar regulatory pathways involving Noggin, Shh, and VEGF have been revealed.

For the long run, adult neural stem cells may require, in addition to a set of specific niche signals, certain intrinsic factors for self-renewal. Bmi-1 and TLX are both transcriptional regulators that are expressed in the nervous system. Interestingly, mice null for either Bmi-1 (Molofsky et al., 2003) or TLX (Shi et al., 2004) have decreased proliferation of postnatal or adult neural stem cells without having severe embryonic neural histogenic phenotypes. The data thus suggest that these intrinsic factors have a specific role in adult neural stem cell maintenance. Perhaps neural phenotypes for these null animals are not very notable in the embryo because of the transient nature of stem cell expansion in development. A similar explanation may hold for the late phenotype of $Smo^{n/c};N^{cre}$ mice (Machold et al., 2003). Hence, while there will likely be many parallels between adult brain germinal zones and embryonic development in both the composition of the niche and identity of stem cell intrinsic factors, certain gene functions may be more clearly manifested in adult stem cells. After all, it is these cells that require a durable mechanism for self-renewal due to the extended period of time that they need to serve as primary precursors.

Concluding Remarks

Ramon y Cajal suggested that neuroepithelial cells are the support elements in the early brain. In the early neural tube, neuroepithelial cells are the only cell type and therefore serve as stem cells as well as "niche cells" that provide signals critical for stem cell function. Astrocytes, which are the "support" elements of the adult brain, appear much later, and Cajal thought of them as "nothing more than displaced and modified neuroepithelial cells." Similar to early neuroepithelial cells, it appears that SVZ/SGZ astrocytes also serve this dual role as stem cells and niche cells. The niche for adult neurogenesis may be nothing more than an early epithelial-like environment, a "pocket" that retains a set of developmental signals that allow some astrocytes to behave as stem cells.

Selected Reading

Capela, A., and Temple, S. (2002). Neuron *35*, 865–875.

Chambers, C.B., Peng, Y., Nguyen, H., Gaiano, N., Fishell, G., and Nye, J.S. (2001). Development *128*, 689–702.

Conover, J.C., Doetsch, F., Garcia-Verdugo, J.M., Gale, N.W., Yancopoulos, G.D., and Alvarez-Buylla, A. (2000). Nat. Neurosci. *3*, 1091–1097.

Doetsch, F., Caille, I., Lim, D.A., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (1999). Cell *97*, 703–716.

Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (2002). Neuron *36*, 1021–1034.

Gage, F.H. (2000). Science *287*, 1433–1438.

Gaiano, N., Nye, J.S., and Fishell, G. (2000). Neuron *26*, 395–404.

Imura, T., Kornblum, H.I., and Sofroniew, M.V. (2003). J. Neurosci. *23*, 2824–2832.

Jin, K., Zhu, Y., Sun, Y., Mao, X.O., Xie, L., and Greenberg, D.A. (2002). Proc. Natl. Acad. Sci. USA *99*, 11946–11950.

Kondo, T., and Raff, M. (2000). Science *289*, 1754–1757.

Lai, K., Kaspar, B.K., Gage, F.H., and Schaffer, D.V. (2003). Nat. Neurosci. *6*, 21–27.

Laywell, E.D., Rakic, P., Kukekov, V.G., Holland, E.C., and Steindler, D.A. (2000). Proc. Natl. Acad. Sci. USA *97*, 13883–13888.

Lim, D.A., and Alvarez-Buylla, A. (1999). Proc. Natl. Acad. Sci. USA *96*, 7526–7531.

Lim, D.A., Tramontin, A.D., Trevejo, J.M., Herrera, D.G., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (2000). Neuron *28*, 713–726.

Machold, R., Hayashi, S., Rutlin, M., Muzumdar, M.D., Nery, S., Corbin, J.G., Gritli-Linde, A., Dellovade, T., Porter, J.A., Rubin, L.L., et al. (2003). Neuron *39*, 937–950.

Mercier, F., Kitasako, J.T., and Hatton, G.I. (2002). J. Comp. Neurol. *451*, 170–188.

Molofsky, A.V., Pardal, R., Iwashita, T., Park, I.K., Clarke, M.F., and Morrison, S.J. (2003). Nature *425*, 962–967.

Nakashima, K., Takizawa, T., Ochiai, W., Yanagisawa, M., Hisatsune, T., Nakafuku, M., Miyazono, K., Kishimoto, T., Kageyama, R., and Taga, T. (2001). Proc. Natl. Acad. Sci. USA *98*, 5868–5873.

Nunes, M.C., Roy, N.S., Keyoung, H.M., Goodman, R.R., McKhann, G., 2nd, Jiang, L., Kang, J., Nedergaard, M., and Goldman, S.A. (2003). Nat. Med. *9*, 439–447.

Palmer, T.D., Willhoite, A.R., and Gage, F.H. (2000). J. Comp. Neurol. *425*, 479–494.

Reynaud-Deonauth, S., Zhang, H., Afouda, A., Taillefert, S., Beatus, P., Kloc, M., Etkin, L.D., Fischer-Lougheed, J., and Spohr, G. (2002). Differentiation *69*, 198–208.

Seri, B., Garcia-Verdugo, J.M., McEwen, B.S., and Alvarez-Buylla, A. (2001). J. Neurosci. *21*, 7153–7160.

Shi, Y., Chichung Lie, D., Taupin, P., Nakashima, K., Ray, J., Yu, R.T., Gage, F.H., and Evans, R.M. (2004). Nature *427*, 78–83.

Song, H., Stevens, C.F., and Gage, F.H. (2002). Nature *417*, 39–44.

Stump, G., Durrer, A., Klein, A.L., Lutolf, S., Suter, U., and Taylor, V. (2002). Mech. Dev. *114*, 153–159.

Tanigaki, K., Nogaki, F., Takahashi, J., Tashiro, K., Kurooka, H., and Honjo, T. (2001). Neuron *29*, 45–55.

Tropepe, V., Craig, C.G., Morshead, C.M., and van der Kooy, D. (1997). J. Neurosci. *17*, 7850–7859.

Ying, Q.L., Nichols, J., Chambers, I., and Smith, A. (2003). Cell *115*, 281–292.