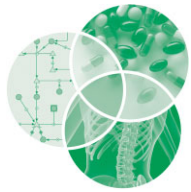
Focus Article



Maintenance and differentiation of neural stem cells

Katlin B. Massirer,[1] Cassiano Carromeu,[1] Karina Griesi-Oliveira[1,2] and Alysson R. Muotri[1]*

The adult mammalian brain contains self-renewable, multipotent neural stem cells (NSCs) that are responsible for neurogenesis and plasticity in specific regions of the adult brain. Extracellular matrix, vasculature, glial cells, and other neurons are components of the niche where NSCs are located. This surrounding environment is the source of extrinsic signals that instruct NSCs to either self-renew or differentiate. Additionally, factors such as the intracellular epigenetics state and retrotransposition events can influence the decision of NSC's fate into neurons or glia. Extrinsic and intrinsic factors form an intricate signaling network, which is not completely understood. These factors altogether reflect a few of the key players characterized so far in the new field of NSC research and are covered in this review. © 2010 John Wiley & Sons, Inc. *WIREs Syst Biol Med*

Adult neural stem cells (NSCs) have the capacity to self-renew and to differentiate into neurons and glia (mainly, astrocytes and oligodendrocytes). In the developed nervous system, NSCs which, likely originated from carryover embryonic cells from the neural plate, are found in the adult hippocampus and in the lateral ventricle.[1,2] Undifferentiated NSCs can then differentiate into neuronal progenitor cells that migrate to more specific areas in the brain, where the newborn neurons integrate and participate in the local network.[3,4] The choice between a multipotent state and a committed state is dictated by changes in the NSC transcriptional program in response to both extrinsic and intrinsic factors. The neurogenic niche and the cellular epigenetic state are some of these factors that lead to transcriptional modifications, which represent the earliest stage of fate commitment. Cells that are committed to the neuronal lineage will change their epigenetic markers by modifying their chromatin state, allowing expression of neuronal-specific genes.[5] Interestingly, the specific transcriptional machinery that activates genes involved in neuronal maturation can also activate a class of endogenous retrotransposons in the genome, previously regarded as 'junk' or 'transcriptional noise'.[6] These retrotransposons are able to insert new copies of themselves into the genome by an endonuclease and reverse transcriptase activity.[7,8] As a consequence, the new insertions can modify the expression of nearby genes, giving rise to slightly different cells with a unique genomic and transcriptional signature.[6,9] Extrinsic and intrinsic factors act in synchrony, granting different layers of plasticity and the necessary complexity for the function of the central nervous system (CNS).

NEUROGENIC NICHE

The niche where adult NSCs reside is a microenvironment that ensures the maintenance of self-renewal and the multipotent state of these cells. This intricate and well-tuned signaling network regulates the transcriptional profile of the NSCs to either keep cells in an undifferentiated state or trigger the precise timing for neurogenesis to occur.

The two niches of neurogenesis in the adult mammalian brain are the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus.[10] In both adult neurogenic zones, the NSCs are in close contact with endothelial cells, astrocytes, ependymal cells, neurons and derived progenitor cells.[10] All these cells have a fundamental role in the preservation of the NSCs' niche homeostasis. Due to the fact that adult neurogenesis was observed specifically in these areas of the

*Correspondence to: muotri@ucsd.edu

[1]Department of Pediatrics/Rady Children's Hospital San Diego, Department of Cellular and Molecular Medicine, University of California San Diego, San Diego, CA, USA

[2]Department of Genetics and Evolutional Biology, Biosciences Institute, University of Sao Paulo, Sao Paulo, Brazil

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brain, most of the initial NSC research has focused on defining the histology and cell biology of these stem cell niches. It was observed that in the SVZ, the relatively quiescent and glial fibrillary acidic protein (GFAP)-positive NSCs can give rise to a more rapidly dividing population of transient amplifying progenitor (TAP) cells, which are GFAP-negative and express the homeobox proteins Dlx2 and epidermal growth factor receptor. A third cell population, composed of neuroblasts generated from TAP cells, migrates through the glial tubes to the olfactory bulb, where they differentiate mainly into inhibitory interneurons.[4,11,12] The quiescent cells are intercalated with ependymal cells that form a monolayer lining the lateral ventricle. Additionally, these cells have an apical process that makes contact with the ventricle wall and a basal process that touches the adjacent vasculature.[13,14] In this way, NSCs can be exposed to a variety of factors circulating in blood vessels or in cerebrospinal fluid, providing nutrients and signaling molecules to start the process of differentiation. Similar to the SVZ, two distinct populations of NSCs reside in the SGZ. Nonradial cells, which proliferate faster and are GFAP-negative, unlike radial cells that are GFAP-positive, do not divide so frequently and have longer processes that contact the granule cell layer. Radial cells may give rise to neuroblasts that migrate to the granule cell layer. Some of these precursor cells will survive and differentiate into excitatory granule neurons and integrate the hippocampus neuronal network.[15,16]

The importance of astrocytes in the NSC niche was first elucidated using coculture experiments with adult-derived NSCs and astrocytes isolated from different regions of the brain. Astrocytes, isolated from neurogenic regions of the CNS, were able to induce NSCs to differentiate into neurons. However, astrocytes from nonneurogenic regions did not stimulate neuronal differentiation, but instead, they promoted the differentiation of NSC into other cell types in culture.[17–19] A key factor released by astrocytes, and involved in this process, is the proto-oncogene Wnt3. The expression of Wnt3 persists in the adult hippocampus and can induce neurogenesis.[20] Additional evidence for the importance of astrocytes comes from experiments inhibiting their capacity to secrete neurogenic factors, which results in decreased differentiation and proliferation of NSCs.[21] A high-throughput proteomic analysis, comparing factors in the medium of secretion-inhibited and noninhibited astrocytes, identified 60 proteins with differential levels, from which 7 are involved in NSC proliferation or differentiation.[21]

A network of blood vessels surrounding the SVZ also represents a crucial component of the NSC niche. The cellular adhesion between the NSC and endothelial cells, which is mediated by laminin receptors in the NSCs, contributes to the proliferation and NSC positioning *in vivo*.[13] The contact between these two types of cells is permeable, allowing soluble molecules in the blood to easily reach NSCs.[13,22] Furthermore, oxygen- and glucose-deprived conditioned medium from endothelial cells promotes neuroblasts' migration and differentiation into neurons.[23] This observation represents strong evidence that endothelial cells release important factors for NSC maintenance and differentiation. Factors such as vascular endothelial growth factor, which induces both angiogenesis and neurogenesis, and pigment epithelium-derived factor (PEDF) are also released by the endothelial cells and are both known to play a role in NSCs' self-renewal.[24,25]

Ependymal cells represent another cellular component of the NSC niche. The release of PEDF by these cells contributes to further regulation of NSC's self-renewal, while the production of the secreted polypeptide Noggin can increase neurogenesis by blocking gliogenesis through a feedback mechanism.[25,26] Ependymal cells also possess a beating cilium generating a gradient of factors involved in neuroblast migration.[27] Finally, neurons in the SVZ and SGZ also participate in the regulation of the niche. They release various neurotransmitters, such as γ-aminobutyric acid, serotonin, dopamine, and glutamate, which can promote differentiation and/or control the proliferation of progenitor cells (for a review, see Ref 2).

NSC EPIGENETICS

While extrinsic factors for NSC differentiation are provided by the niche, intrinsic factors comprise mainly DNA sequences and epigenetic modifications.[28,29] Epigenetics is defined as any structural modification of genomic regions that leads to a change in gene expression. Such modifications may be heritable through the process of meiosis or mitosis, without changes in the DNA sequence.[30] The four major mechanisms of epigenetic regulation are DNA methylation, chromatin remodeling (by covalent modification of histone tails), noncoding RNA (ncRNA) regulation, and RNA editing.[31–36]

During NSC differentiation, cells change their internal program from the self-renewal state to a committed fate. This transition is synchronized with a global alteration of the transcriptome.[29] The genome of precursor cells is maintained in a poised state, where important developmental genes have both repressor and activator transcription markers in their regulatory promoter regions.[37,38] When

differentiation is activated, specific cell fate genes are upregulated by activator markers, while repressor markers are lost.[39–42] A poised state allows an immediate transcriptional response upon extrinsic and intrinsic signals.

The epigenetic markers dictating transcriptional activity are mainly controlled by histone modification and DNA methylation. DNA methylation occurs most commonly on CpG dinucleotides, generally leading to transcriptional silencing of the target gene.[28] Several proteins mediate such gene silencing. One well-studied example is the methyl CpG binding protein 2 (MeCP2). MeCP2 preferentially binds methylated DNA to regulate transcription, usually leading to gene repression.[43]

In the poised NSC state, the synchrony between expression of specific transcription factors (TFs) and epigenetic modifications can be exemplified by the neuron-restrictive silencing factor/RE-1 silencing transcription (REST) factor. This system utilizes a scaffold of large complexes, required for all stages of differentiation from precursors to neurons.[44–47] REST binds to RE-1 sequences present in promoters of various neuronal genes.[28] The complex formed from the protein–DNA interactions leads to transcriptional silence of these regions in NSC. Acting together with the chromatin architecture and methylation status, TFs dictate the specificity and timing of gene transcription.

ncRNAs have recently been proposed to exert a new layer of epigenetic control. In addition to coding genes, REST appears to control the expression of diverse miRNAs (one class of ncRNAs), including mir-9, mir-124a, and mir-132.[48] Interestingly, mir-9 seems to form a negative feedback loop with REST, targeting the REST sequence.[49] In addition, mir-132 influences this dynamic by targeting MeCP2, which interacts with REST to suppress transcription.[50] Regulation of intracellular levels of miRNAs can interfere with the current transcriptome program. The importance of miRNAs in neurogenesis can be illustrated by mir-124a, a brain-specific miRNA that accounts for nearly half of all brain miRNAs.[51] mir-124a expression peaks during neurogenesis and persists in mature neurons.[35,36] The overexpression of mir-124a in non-neural cells changes the expression of these cells to a more neuron-like profile.[52]

Once activated, the fate program alters epigenetic markers, inducing chromatin remodeling. The accessibility to chromatin allows cell specification signals, TFs for instance, to interact with target sequences.[53] For example, the basic helix–loop–helix TF Ascl1 (also known as Mash1) is inactive in NSCs and its expression is tightly regulated during development.[54,55] Mammalian hairy and enhancer of split homologue (HES1), a transcriptional repressor activated in Notch signaling, forms a complex with transducin-like enhancer of split 1 (TLE1) and inhibits Ascl1, recruiting histone deacetylase (HDAC) and deacetylase subunit SIN3a.[54] The Ascl1 locus is at the nuclear periphery and replicates late during S phase in NSCs.[56] This late replication time is correlated with packed chromatin and transcriptional silencing.[57,58] However, upon neuronal differentiation stimulus, Ascl1 relocates inside the nucleus. Poly(ADP-ribose) polymerase (PARP-1) is also present in the repressor complex HES1/TLE1. PARP-1 works as a sensor of niche PDGF signaling and acts by dismissing TLE1 from the repressor complex and modifying HES1. This modification switches the function of HES1 from repressor to activator, recruiting histone acetylase proteins and modifying access to chromatin.[59] Altogether, these modifications lead to activation of Ascl1.

In vivo, the Ascl1 gene seems to have different contributions depending on the cellular niche (Figure 1). In the SVZ, the Ascl1 gene is highly expressed in progenitor cells, driving differentiation to neurons. However, in the SGZ, Ascl1 expression is very low in cells originating neurons. Indeed, the overexpression of Ascl1 in progenitor cells from the SGZ leads to a fate change, driving cell differentiation toward oligodendrocytes.[60] Interestingly, the same effect was not observed *in vitro*. SGZ progenitors generate neurons even when Ascl1 is overexpressed in tissue culture (Figure 1). This feature points to the importance of the niche–epigenome interaction as a crucial factor determining cell fate.

Differentiation from a progenitor state toward neurons causes dramatic changes in the chromatin architecture landscape, with neurons having a more compact heterochromatin.[5,61] These changes include the silencing of genes committed to non-neuronal fate. In addition, the niche induces a signal transduction network through plasma membrane receptors that will influence the TFs milieu and their action in the nucleus.[62] The final decision concerning which genes are activated and which are repressed relies on the interactions of these TFs with the epigenetic status of the genome at a specific time point (Figure 2).

RETROTRANSPOSITION AND NEUROGENESIS

Long interspersed nuclear elements 1 (LINE-1 or L1) are discrete repetitive and retrotransposable sequences found in the genome of most eukaryotes. L1s may contribute to another facet of NSC

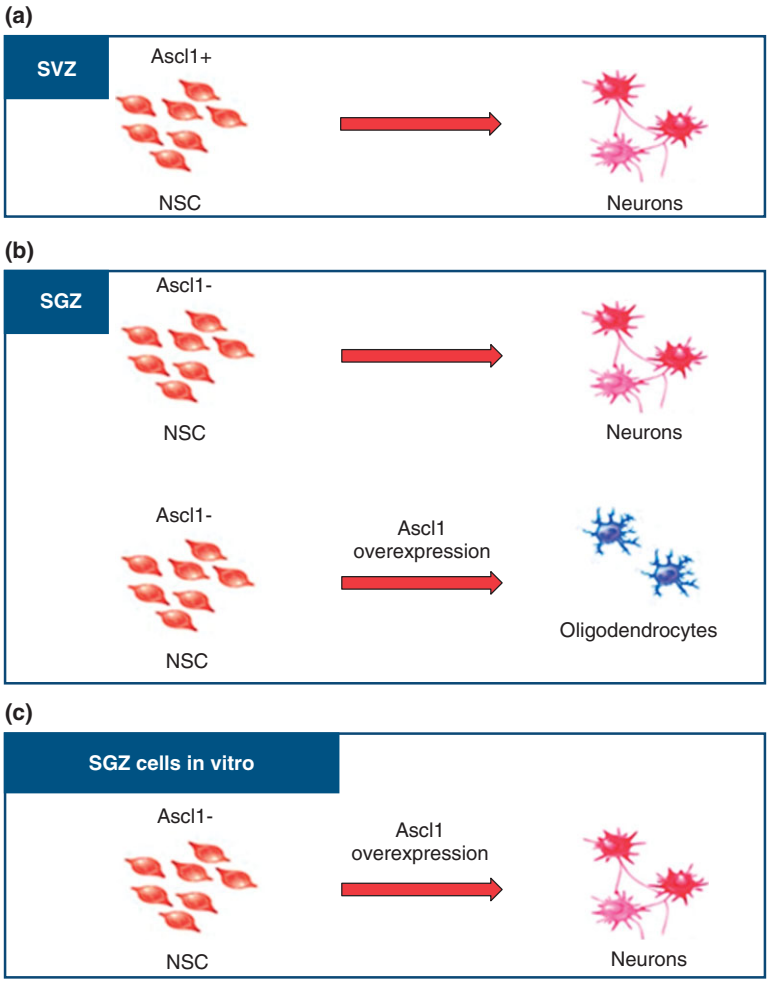

FIGURE 1 | Combinatorial influence of niche factors and epigenetics in the decision on neuronal fate. Neural stem cells (NSC) from the subventricular zone committed to becoming neurons express the Ascl1 gene (A). On the contrary, NSCs from the subgranular zone (SGZ) show low Ascl1 expression (B). *In vivo* overexpression of Ascl1 in the same SGZ cells leads to an oligodendrogenesis (B). However, overexpression of Ascl1 in NSCs from SGZ, cultured *in vitro*, leads to neuronal fate (C).

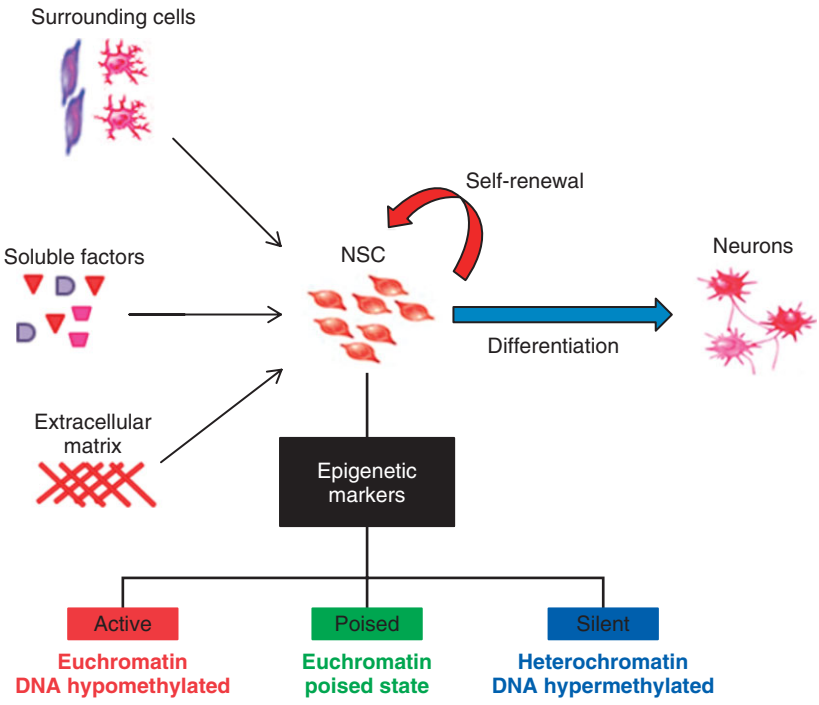

FIGURE 2 | Signaling network regulating neural stem cell (NSC) self-renewal and the decision on cell fate. Dynamics between extrinsic and intrinsic signals defines the NSC's self-renewal or differentiation state.

regulation. L1s constitute about 20% of mammalian genomes, and sequences corresponding to full-length L1s are approximately 6 kb long.[63–65] Active L1s were initially studied in germline but were also recently detected in both rodent and human neuronal progenitor cells.[6,66] Interestingly, a higher number of L1 retrotransposon sequences were observed in samples from human brain or neuronal tissues compared with other somatic tissues, such as heart and liver.[67] These observations raised the question of an eventual but specific contribution of transposition in the nervous system. Generally, most L1 elements are transcriptionally inactive due to 5′ truncations, internal rearrangements, and mutations in their open-reading frames.[68] Nevertheless, among all full-length L1 copies in the genome, only about 150 are considered active in humans.[63]

Several extrinsic signaling and epigenetic factors related to neuronal differentiation have been associated with the activation of L1 retrotransposition. Luciferase experiments using the human endogenous L1 5′ UTR promoter region lead to the observation that early stages of neuronal differentiation correlate with L1 transcriptional activation.[6] Further *in vitro* investigation using adult rat neuroprogenitor cells and *in vivo* mouse brain demonstrated that retrotransposition events could affect the expression of targeted neuronal genes such as postsynaptic density 93.[6]

Studies on L1 expression during neurogenesis led to the finding of binding sites for the neuronal TF Sox2 in human L1 5′ UTR.[6,69] Initially, experiments inducing neuronal differentiation from NSCs resulted in upregulation of L1 expression, while Sox2 was downregulated.[6,70] More specific characterization of Sox2 binding sites by chromatin immunoprecipitation experiments showed the occupancy of these sites within the endogenous L1 promoter in undifferentiated cells (Figure 3). This association was lost after neuronal differentiation. Additionally, HDAC1 was also associated with L1 promoter in undifferentiated cells, indicating that recruitment of various factors is necessary to keep L1 transcriptionally silenced. Interestingly, the expression and transcriptional mechanism of L1 mirrors the activation of the TF NeuroD1, which also contains Sox2 bound to its promoter in the NSC state.[70]

It was recently shown that Wnt3 can induce neurogenesis by activating NeuroD1.[70] This mechanism involves the canonical Wnt pathway, with cytoplasmic translocation of T cell factor/lymphoid enhancer factor (TCF/LEF) to the nucleus (Figure 3). Unexpectedly, TCF/LEF also induces L1 retrotransposon transcription.[70] These observations led to the conclusion that Wnt activation of L1 is coordinated with NeuroD1 activation during neurogenesis. Further studies on an increased number of neuronal genes will elucidate whether retrotransposition could have a broad effect on neurogenesis.

Because accessible chromatin during neuronal differentiation comprises regions rich in neuronal genes, an open-chromatin state represents potential target regions for *de novo* L1 integration. Insertions in the open chromatin can lead to different transcriptional signatures in distinct neurons. Advances in the understanding of L1 retrotransposition biology during neuronal differentiation will help to elucidate the

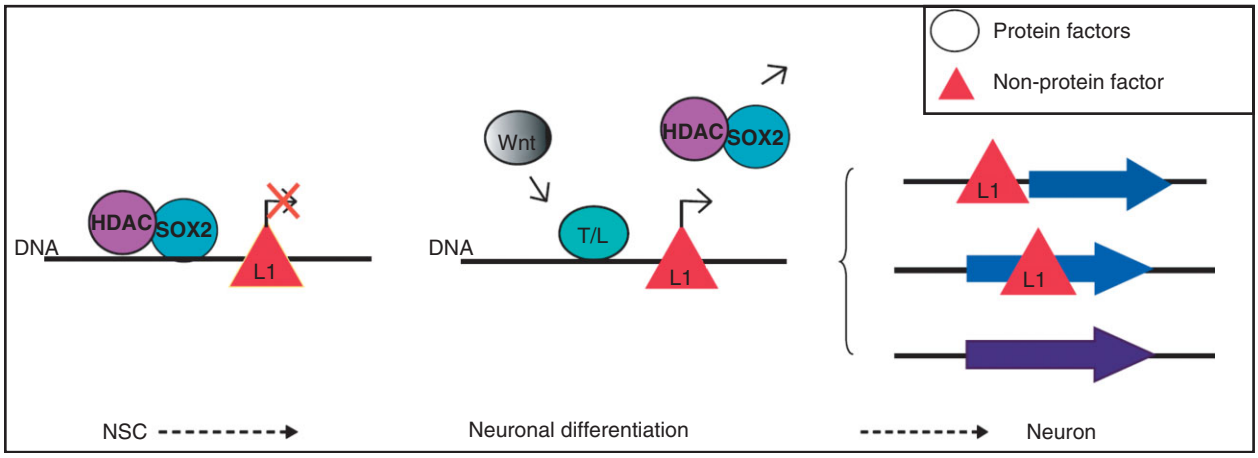

FIGURE 3 | Retrotransposition activation during neuronal differentiation. Long interspersed nuclear elements 1 (L1) are inhibited in neural stem cells by condensed chromatin and Sox2/histone deacetylase 1 repression. During neuronal differentiation, the chromatin is remodeled from a repressed to an activated state. At this stage, Wnt released from astrocytes stimulate the TCF/LEF (T/C) translocation from the cytoplasm to the nucleus. TCF/LEF-binding sites overlap with Sox2-binding sites in several genomic regions, including the promoter region of L1s. As a consequence of L1 expression, *de novo* integrations can occur in different genomic regions and potentially affect target gene expression.

intricate cross-talk between exogenous niche signals and endogenous epigenetic control.

CONCLUSIONS AND PERSPECTIVES

The presence of NSCs and their ability to differentiate in newborn neurons in the adult brain can be viewed as one of the major paradigm shifts in neurosciences. The components described in this review illustrate the uniqueness of the cellular architecture and the complexity of the brain. Recent advances in NSC culture technology will allow the identification of new players in fundamental processes involved in neuronal differentiation. In addition, *in vivo* experiments will contribute to more detailed analyses of the cellular niche and its interactions. Moreover, the possibility of reprogramming adult somatic cells from patients with neurological diseases is broadening the spectrum of phenotypes that can be evaluated in a dish. The interaction of niche–epigenome–ncRNA seems to be synergistic in the brain, defining cellular self-renewal and differentiation. As a result, mobile elements can be activated, generating a heterogeneous neuronal population important for brain functionality and plasticity. Undoubtedly, the knowledge generated from these experiments will expand possibilities for future regenerative therapies.

REFERENCES

1. Johansson CB, Svensson M, Wallstedt L, Janson AM, Frisen J. Neural stem cells in the adult human brain. *Exp Cell Res* 1999, 253:733–736.
2. Suh H, Deng W, Gage FH. Signaling in adult neurogenesis. *Annu Rev Cell Dev Biol* 2009, 25:253–275.
3. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 1999, 97:703–716.
4. Quinones-Hinojosa A, Sanai N, Soriano-Navarro M, Gonzalez-Perez O, Mirzadeh Z, Gil-Perotin S, Romero-Rodriguez R, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. *J Comp Neurol* 2006, 494:415–434.
5. Takizawa T, Meshorer E. Chromatin and nuclear architecture in the nervous system. *Trends Neurosci* 2008, 31:343–352.
6. Muotri AR, Chu VT, Marchetto MC, Deng W, Moran JV, Gage FH. Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. *Nature* 2005, 435:903–910.
7. Feng Q, Moran JV, Kazazian HH, Jr., Boeke JD. Human L1 retrotransposon encodes a conserved endonuclease required for retrotransposition. *Cell* 1996, 87:905–916.
8. Mathias SL, Scott AF, Kazazian HH, Jr., Boeke JD, Gabriel A. Reverse transcriptase encoded by a human transposable element. *Science* 1991, 254:1808–1810.
9. Mehler MF. Epigenetics and the nervous system. *Ann Neurol* 2008, 64:602–617.
10. Ma DK, Bonaguidi MA, Ming GL, Song H. Adult neural stem cells in the mammalian central nervous system. *Cell Res* 2009, 19:672–682.
11. Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci* 1997, 17:5046–5061.
12. Kokovay E, Shen Q, Temple S. The incredible elastic brain: how neural stem cells expand our minds. *Neuron* 2008, 60:420–429.
13. Shen Q, Wang Y, Kokovay E, Lin G, Chuang SM, Goderie SK, Roysam B, Temple S. Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* 2008, 3:289–300.
14. Mirzadeh Z, Merkle FT, Soriano-Navarro M, Garcia-Verdugo JM, Alvarez-Buylla A. Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell* 2008, 3:265–278.
15. Seri B, Garcia-Verdugo JM, Collado-Morente L, McEwen BS, Alvarez-Buylla A. Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J Comp Neurol* 2004, 478:359–378.
16. Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. *Cell* 2008, 132:645–660.
17. Song HJ, Stevens CF, Gage FH. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat Neurosci* 2002, 5:438–445.
18. Song H, Stevens CF, Gage FH. Astroglia induce neurogenesis from adult neural stem cells. *Nature* 2002, 417:39–44.
19. Lim DA, Alvarez-Buylla A. Interaction between astrocytes and adult subventricular zone precursors stimulates neurogenesis. *Proc Natl Acad Sci U S A* 1999, 96:7526–7531.
20. Lie DC, Colamarino SA, Song HJ, Desire L, Mira H, Consiglio A, Lein ES, Jessberger S, Lansford H, Dearie

AR, et al. Wnt signalling regulates adult hippocampal neurogenesis. *Nature* 2005, 437:1370–1375.

21. Yan H, Zhou W, Wei L, Zhong F, Yang Y. Proteomic analysis of astrocytic secretion that regulates neurogenesis using quantitative amine-specific isobaric tagging. *Biochem Biophys Res Commun* 2010, 391:1187–1191.
22. Tavazoie M, Van der Veken L, Silva-Vargas V, Louissaint M, Colonna L, Zaidi B, Garcia-Verdugo JM, Doetsch F. A specialized vascular niche for adult neural stem cells. *Cell Stem Cell* 2008, 3:279–288.
23. Plane JM, Andjelkovic AV, Keep RF, Parent JM. Intact and injured endothelial cells differentially modulate postnatal murine forebrain neural stem cells. *Neurobiol Dis* 2010, 37:218–227.
24. Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, During MJ. VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet* 2004, 36:827–835.
25. Ramirez-Castillejo C, Sanchez-Sanchez F, Andreu-Agullo C, Ferron SR, Aroca-Aguilar JD, Sanchez P, Mira H, Escribano J, Farinas I. Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. *Nat Neurosci* 2006, 9:331–339.
26. Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A. Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 2000, 28:713–726.
27. Sawamoto K, Wichterle H, Gonzalez-Perez O, Cholfin JA, Yamada M, Spassky N, Murcia NS, Garcia-Verdugo JM, Marin O, Rubenstein JL, et al. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* 2006, 311:629–632.
28. Hamby ME, Coskun V, Sun YE. Transcriptional regulation of neuronal differentiation: the epigenetic layer of complexity. *Biochim Biophys Acta* 2008, 1779:432–437.
29. Mehler MF. Epigenetic principles and mechanisms underlying nervous system functions in health and disease. *Prog Neurobiol* 2008, 86:305–341.
30. Bird A. Perceptions of epigenetics. *Nature* 2007, 447:396–398.
31. Mehler MF, Mattick JS. Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. *Physiol Rev* 2007, 87:799–823.
32. Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* 2007, 8:355–367.
33. Feng J, Fouse S, Fan G. Epigenetic regulation of neural gene expression and neuronal function. *Pediatr Res* 2007, 61:58R–63R.
34. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 2006, 31:89–97.
35. Kondo T. Epigenetic alchemy for cell fate conversion. *Curr Opin Genet Dev* 2006, 16:502–507.
36. Taniura H, Iijima S, Kambe Y, Georgiev D, Yoneda Y. Tex261 modulates the excitotoxic cell death induced by *N*-methyl-D-aspartate (NMDA) receptor activation. *Biochem Biophys Res Commun* 2007, 362:1096–1100.
37. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 2007, 448:553–560.
38. Kageyama R, Nakanishi S. Helix-loop-helix factors in growth and differentiation of the vertebrate nervous system. *Curr Opin Genet Dev* 1997, 7:659–665.
39. Azuara V. Profiling of DNA replication timing in unsynchronized cell populations. *Nat Protoc* 2006, 1:2171–2177.
40. Spivakov M, Fisher AG. Epigenetic signatures of stem-cell identity. *Nat Rev Genet* 2007, 8:263–271.
41. Gaspar-Maia A, Alajem A, Polesso F, Sridharan R, Mason MJ, Heidersbach A, Ramalho-Santos J, McManus MT, Plath K, Meshorer E, et al. Chd1 regulates open chromatin and pluripotency of embryonic stem cells. *Nature* 2009, 460:863–868.
42. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 2006, 125:315–326.
43. Jorgensen HF, Azuara V, Amoils S, Spivakov M, Terry A, Nesterova T, Cobb BS, Ramsahoye B, Merkenschlager M, Fisher AG. The impact of chromatin modifiers on the timing of locus replication in mouse embryonic stem cells. *Genome Biol* 2007, 8:R169.
44. Ballas N, Mandel G. The many faces of REST oversee epigenetic programming of neuronal genes. *Curr Opin Neurobiol* 2005, 15:500–506.
45. Ooi L, Wood IC. Chromatin crosstalk in development and disease: lessons from REST. *Nat Rev Genet* 2007, 8:544–554.
46. Otto SJ, McCorkle SR, Hover J, Conaco C, Han JJ, Impey S, Yochum GS, Dunn JJ, Goodman RH, Mandel G. A new binding motif for the transcriptional repressor REST uncovers large gene networks devoted to neuronal functions. *J Neurosci* 2007, 27:6729–6739.
47. Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S. REST maintains self-renewal and pluripotency of embryonic stem cells. *Nature* 2008, 453:223–227.
48. Conaco C, Otto S, Han JJ, Mandel G. Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc Natl Acad Sci U S A* 2006, 103:2422–2427.

49. Packer AN, Xing Y, Harper SQ, Jones L, Davidson BL. The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. *J Neurosci* 2008, 28:14341–14346.
50. Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH. Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. *Nat Neurosci* 2007, 10:1513–1514.
51. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. *Curr Biol* 2002, 12:735–739.
52. Lim LP Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005, 433:769–773.
53. Lessard J, Wu JI, Ranish JA, Wan M, Winslow MM, Staahl BT, Wu H, Aebersold R, Graef IA, Crabtree GR. An essential switch in subunit composition of a chromatin remodeling complex during neural development. *Neuron* 2007, 55:201–215.
54. Kageyama R, Ohtsuka T, Hatakeyama J, Ohsawa R. Roles of bHLH genes in neural stem cell differentiation. *Exp Cell Res* 2005, 306:343–348.
55. Bertrand N, Castro DS, Guillemot F. Proneural genes and the specification of neural cell types. *Nat Rev Neurosci* 2002, 3:517–530.
56. Williams RR, Azuara V, Perry P, Sauer S, Dvorkina M, Jorgensen H, Roix J, McQueen P, Misteli T, Merkenschlager M, et al. Neural induction promotes large-scale chromatin reorganisation of the Mash1 locus. *J Cell Sci* 2006, 119:132–140.
57. Gilbert DM. Replication timing and transcriptional control: beyond cause and effect. *Curr Opin Cell Biol* 2002, 14:377–383.
58. Schubeler D, Scalzo D, Kooperberg C, van Steensel B, Delrow J, Groudine M. Genome-wide DNA replication profile for Drosophila melanogaster: a link between transcription and replication timing. *Nat Genet* 2002, 32:438–442.
59. Ju BG, Solum D, Song EJ, Lee KJ, Rose DW, Glass CK, Rosenfeld MG. Activating the PARP-1 sensor component of the groucho/TLE1 corepressor complex mediates a CaMKinase IIdelta-dependent neurogenic gene activation pathway. *Cell* 2004, 119:815–829.
60. 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.
61. Meshorer E, Misteli T. Chromatin in pluripotent embryonic stem cells and differentiation. *Nat Rev Mol Cell Biol* 2006, 7:540–546.
62. Lunyak VV, Rosenfeld MG. Epigenetic regulation of stem cell fate. *Hum Mol Genet* 2008, 17:R28–R36.
63. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, et al. Initial sequencing and analysis of the human genome. *Nature* 2001, 409:860–921.
64. Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, Scott G, Steffen D, Worley KC, Burch PE, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* 2004, 428:493–521.
65. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexandersson M, An P, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002, 420:520–562.
66. Branciforte D, Martin SL. Developmental and cell type specificity of LINE-1 expression in mouse testis: implications for transposition. *Mol Cell Biol* 1994, 14:2584–2592.
67. Coufal NG, Garcia-Perez JL, Peng GE, Yeo GW, Mu Y, Lovci MT, Morell M, O'Shea KS, Moran JV, Gage FH. L1 retrotransposition in human neural progenitor cells. *Nature* 2009, 460:1127–1131.
68. Ostertag EM, Kazazian HH Jr. Biology of mammalian L1 retrotransposons. *Annu Rev Genet* 2001, 35:501–538.
69. Tchenio T, Casella JF, Heidmann T. Members of the SRY family regulate the human LINE retrotransposons. *Nucleic Acids Res* 2000, 28:411–415.
70. Kuwabara T, Hsieh J, Muotri A, Yeo G, Warashina M, Lie DC, Moore L, Nakashima K, Asashima M, Gage FH. Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. *Nat Neurosci* 2009, 12:1097–1105.