nature neuroscience

Epigenetic choreographers of neurogenesis in the adult mammalian brain

Dengke K Ma[1], Maria Carolina Marchetto[2], Junjie U Guo[3,4], Guo-li Ming[3–5], Fred H Gage[2] & Hongjun Song[3–5]

Epigenetic mechanisms regulate cell differentiation during embryonic development and also serve as important interfaces between genes and the environment in adulthood. Neurogenesis in adults, which generates functional neural cell types from adult neural stem cells, is dynamically regulated by both intrinsic state-specific cell differentiation cues and extrinsic neural niche signals. Epigenetic regulation by DNA and histone modifiers, non-coding RNAs and other self-sustained mechanisms can lead to relatively long-lasting biological effects and maintain functional neurogenesis throughout life in discrete regions of the mammalian brain. Here, we review recent evidence that epigenetic mechanisms carry out diverse roles in regulating specific aspects of adult neurogenesis and highlight the implications of such epigenetic regulation for neural plasticity and disorders.

Were Cajal alive today, he would perhaps be surprised to learn that many thousands of new neurons are generated every day in an adult mammalian brain[1]. The dogma that the adult mammalian CNS does not generate new neurons has been overturned[2,3]. Adult neurogenesis, which is broadly defined as a process of generating functional neural cell types from adult neural stem cells, occurs in two discrete areas of the mammalian brain[4–8]. In the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, adult neural stem cells undergo proliferation, fate specification, maturation, migration and eventual integration into the pre-existing neural circuitry[9]. Principal dentate granule cells are the only neuronal subtype that is generated, and newly generated neurons have distinct properties that enable them to contribute to specialized functions in learning and memory[10–12]. In the subventricular zone (SVZ) of the lateral ventricle, adult neural stem cells give rise to glia and neuroblasts[6,7]. These neuroblasts migrate over a long distance to the olfactory bulb and differentiate into local interneurons that have various functions in olfaction.

Adult neurogenesis can be viewed as a classic process of cell differentiation, but it occurs in the unique environment of the mature nervous system. Intrinsically, adult neural stem cells pass through sequential developmental stages that show structurally and functionally distinct cellular properties. As noted by Holiday and Waddington[13–16], who originally coined the term 'epigenetics', cell differentiation during development results essentially from epigenetic changes to identical genomes through temporal and spatial control of gene activity. The process of adult neurogenesis is therefore intrinsically under similarly choreographed epigenetic control. Extrinsically, adult neurogenesis is precisely modulated by a wide variety of environmental, physiological and pharmacological stimuli. At the interface between genes and the environment[17,18], epigenetic mechanisms naturally serve as key conduits for the regulation of adult neurogenesis by the environment, experience and internal physiological states in the form of local or systemic extracellular signaling molecules and patterns of neural circuit activity[19–21].

Epigenetic mechanisms imply cellular processes that do not alter the genomic sequence, and they are believed to elicit relatively persistent biological effects. Processes that can modulate DNA or associated structures independently of the DNA sequence, such as DNA methylation, histone modification, chromatin remodeling and transcriptional feedback loops, are thought to constitute the main epigenetic mechanisms (**Fig. 1a**). DNA methylation at the 5-position of the nucleotide cytosine ring is relatively stable, and the maintenance DNA methyltransferase (Dnmt) ensures its epigenetic inheritance during DNA replication[22,23] (**Fig. 1b**). A newly discovered modification of DNA, hydroxylation of the 5-methyl group, which gives rise to 5-hydroxymethylcytosine, is present in various brain regions[24] and in pluripotent stem cells[25,26], although its biological function remains unknown. Specific amino-acid residues of histone N-terminal tails can be reversibly modified by different mechanisms, such as acetylation, phosphorylation, methylation, ubiquitination, SUMOylation and isomerization (**Fig. 1c**). The varying turnover rates and biological interpreters of these modifications might underpin different epigenetic cellular functions[22,27]. In addition to chromatin-based epigenetic mechanisms, other self-sustaining processes might also be epigenetic in nature, such as prion-mediated perpetuation of protein conformation changes and transcriptional regulator–mediated autoregulatory feedback loops that are long-lasting in the absence of the initial trigger stimuli[28,29]. In proliferating neural stem cells, epigenetic mechanisms can elicit heritable long-lasting effects after many rounds of cell division. In postmitotic newborn neurons or mature neurons, epigenetic mechanisms may produce distinct effects as 'cellular memory' independent of cell division. Importantly, although epigenetic effects are relatively long-lasting, it is changes in epigenetic programs that help to choreograph the precisely timed

[1]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA. [2]Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, California, USA. [3]Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [4]The Solomon Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [5]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. Correspondence should be addressed to H.S. (shongju1@jhmi.edu).

Published online 26 October 2010; doi:10.1038/nn.2672

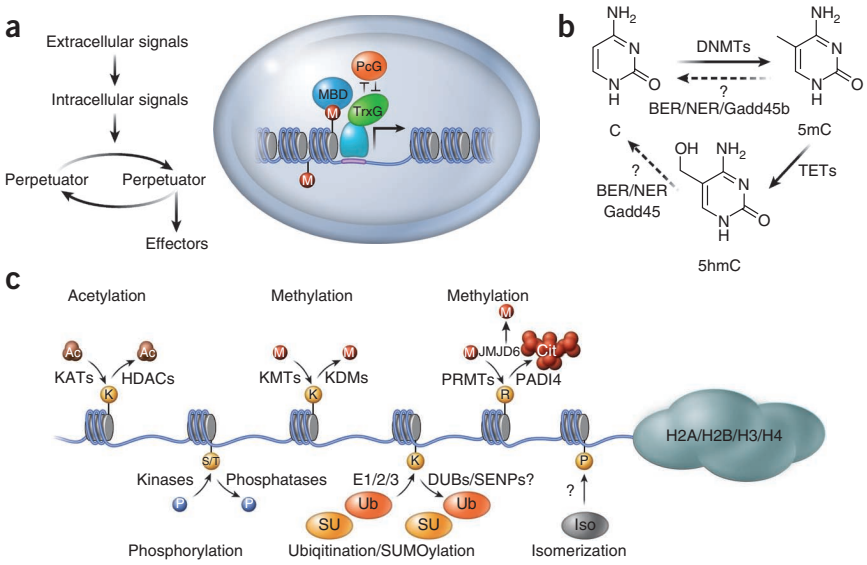

Figure 1 Basic modes of epigenetic regulation implicated in adult neurogenesis. (**a**) To initiate epigenetic processes, extracellular and intracellular signals may trigger epigenetic 'perpetuators' that form self-sustaining feedback loops or intrinsically produce long-lasting cellular effects in the absence of the initial trigger stimuli. Typical mechanisms by which this process occurs include transcription regulator and non-coding RNA–mediated feedback pathways, DNA methylation with associated methyl-binding proteins (MBDs), and histone H3K27 methylation with associated PcG (polycomb group) and TrxG (trithorax group) complexes. (**b**) DNA modifications. DNA methyltransferases (DNMTs) catalyze DNA methylation, whereas the pathway leading to DNA demethylation might include 5-methylcytosine (5mC) hydroxylase TET (ten-eleven translocation-1) proteins and DNA excision repair enzymes that are regulated by Gadd45 (growth arrest and DNA-damage-inducible) family proteins. (**c**) Histone modifications. Specific amino acid residues of histone N-terminal tails can be reversibly modified with a variety of 'tags' including acetylation (ac), phosphorylation (p), methylation (me), ubiquitination (ub), SUMOylation (su) and isomerization (iso). The varying turnover rates and biological interpreters of these modifications might execute different cellular functions for epigenetic regulation. C, cytosine; 5mC, 5-methylcytosines; 5hmC, 5-hydroxymethylcytosine; BER, base-excision repair; NER, nucleotide-excision repair; K, lysine; S, serine; T, threonine; R, arginine; P, proline; KAT, lysine acetyltransferase; HDAC, histone deacetylase; KMT, lysine methyltransferase; KDM, lysine deacetylase; PRMT, protein arginine methyltransferase; PADI4, peptidyl arginine deiminase type IV; JMJD6, Fe(II) and 2-oxoglutarate–dependent dioxygenase Jumonji domain-6 protein; DUB, deubiquitinase; SENP, sentrin-specific protease.

transitions from one cellular state to another in coordination with both internal and external cues during adult neurogenesis.

In this review we first consider important methodological issues in studying the epigenetics of adult neurogenesis. We then discuss key epigenetic mechanisms that regulate specific stages of adult neurogenesis, and conclude by highlighting some general principles and their implications for our understanding of several brain disorders.

Technologies for epigenetic analysis of adult neurogenesis

The study of epigenetics in the context of the adult nervous system involves specific technical challenges. To profile chromatin-based epigenetic modifications, such as DNA methylation or histone modifications, it is usually necessary to analyze a large, relatively homogeneous population of cells, as each diploid cell has only two such gene-specific modifications. Moreover, *in situ* analysis using specific labeling reagents, such as antibodies, is limited to global changes in chromatin modification and lacks gene-specific resolution.

New technologies are now available to help researchers to overcome some of these obstacles (**Fig. 2**). Cells of sequential developmental stages are conventionally identified by immunophenotyping, labeling with thymidine analogs or retroviral infection[5]. Cell-surface antigens that are specific for adult neural progenitors have proven to be effective for flow cytometric isolation and purification[30,31], although more specific antigens for the progeny of adult neural stem cells still need to be developed and the different subclasses of adult neural progenitors defined by these markers require additional functional characterization. Many transgenic reporter mouse lines have been generated in which specific classes of progenitors and newborn neurons are fluorescently labeled[32,33], allowing targeted populations of live cells to be prospectively isolated in large numbers for analysis. In addition, methodologies have been developed to allow intact neuronal nuclei to be isolated from the brain[34]. The combined application of these technologies will be powerful and will make possible unprecedented epigenetic analyses of nearly every developmental stage during adult neurogenesis.

Emerging technologies have also become available for more cost-effective, sensitive, precise and genome-wide scale measurements of epigenetic DNA and histone modifications. The gold standard in DNA methylation analysis has been bisulfite sequencing, in which methylated and unmethylated cytosines are distinguished after conversion of unmethylated but not methylated cytosines into uracils by bisulfite. However, this procedure causes substantial loss of samples and requires both a large amount of starting material and PCR amplification for Sanger-based sequencing. These obstacles have been essentially overcome by the recent development of significantly improved procedures, such as carrier supplementation and more sensitive and accurate bisulfite sequencing methods[35,36]. In addition, restriction enzyme–based quantitative methylation analysis is an independent approach for validation and is less labor-intensive. By taking advantage of next-generation sequencing technologies, researchers can now profile the DNA methylome at the genome scale with single-nucleotide resolution[37,38].

The combined use of chromatin immunoprecipiation (ChIP; a method of profiling chromatin modifications at genomic loci) with RNA expression analysis, as well as new technical adjustments to these techniques, can help to identify biological signatures for activated or repressed chromatin states during adult neurogenesis. In ChIP, specific and effective antibodies are required to pull down chromatin fractions for quantitative detection of the DNA sequence. Variants of ChIP, such as sequential ChIP, can be used to detect the simultaneous presence of multiple histone modifications, such as the bivalent domain (H3K4 and H3K27 methylation) that is typical of genes that are poised for key developmental regulators in progenitor cells[39]. Another powerful variant, ChIP-seq, combines ChIP with next-generation sequencing for genome-wide profiling of histone modifications[40]. ChIP can also be used to profile DNA methylation and remains the method of choice to distinguish between DNA methylation and 5-methylcytosine hydroxylation[24,41].

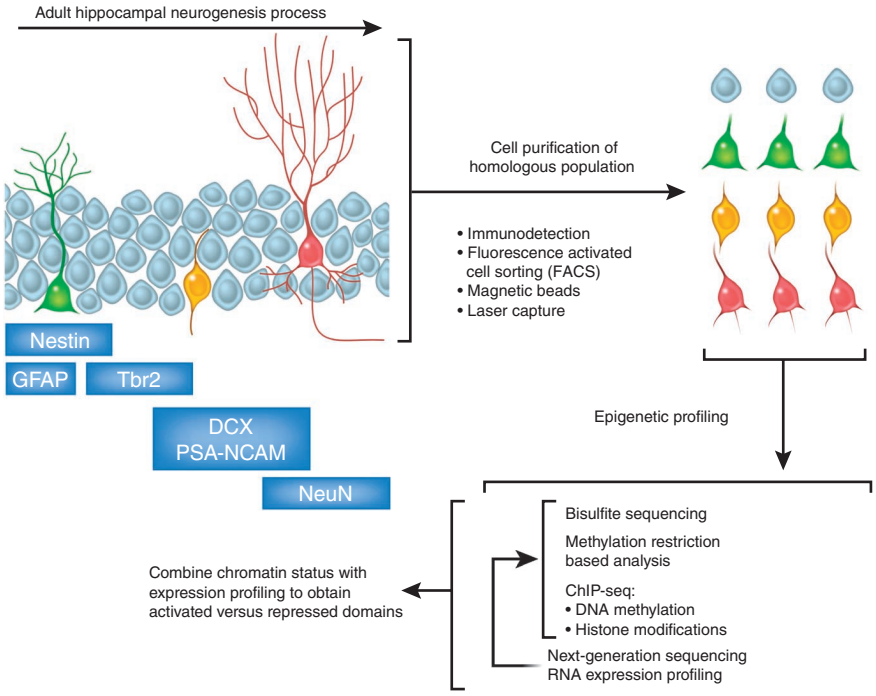

Figure 2 Classic and emerging technologies for epigenetic analysis of adult neurogenesis. During adult neurogenesis in the SGZ, neural stem cells (nestin$^+$ and GFAP$^+$) differentiate into immediate neural progenitors (Trb2$^+$) and then newborn neurons (DCX$^+$ and PSA-NCAM$^+$) and finally into mature new neurons (NeuN$^+$). To profile gene-specific epigenetic modifications, homogeneous target cell populations should be isolated and purified using laser capture microdissection or various prospective cell-labeling strategies. DNA methylation analysis can then be performed using bisulfite sequencing or methylation-sensitive restriction-based approaches. ChIP can be used to profile both DNA and histone modifications. The emerging technology of next-generation sequencing platforms allows rapid, genome-scale, high-resolution mapping of both DNA and histone modifications. RNA expression profiling may validate and further reveal new biological signatures for activated or repressed chromatin states.

Epigenetic regulation of neural stem cell maintenance

Self-renewal and maintenance of developmental potentials are hallmarks of stem cells and are necessary for adult neural stem cells to generate neurons continuously throughout life. There is now evidence that the polycomb group protein Bmi1 is a key epigenetic regulator for these processes[42] (**Fig. 3a**). The polycomb and trithorax group (PcG and TrxG) proteins are antagonistic chromatin complexes that silence or activate their target loci, respectively, and maintain corresponding expression states over many cell divisions[43,44]. Bmi1 is a member of the PcG complex that catalyzes H3K27 methylation, whereas the TrxG complex catalyzes H3K4 methylation. SVZ-derived adult neural stem cells, but not lineage-restricted progenitors, are depleted in Bmi1 knockout mice[42]. The effect of Bmi1 seems to be mediated by the cell cycle inhibitor p16 protein, as mice lacking both Bmi1 and p16 have an almost normal number of adult neural stem cells. Furthermore, Bmi1 overexpression *in vitro* significantly increases the number of adult neural stem cells in the SVZ and maintains their developmental potential to generate neuronal lineages[45,46]. Given that the effect of Bmi1 overexpression *in vivo* might depend on its dose and that Bmi1 is also required for the self-renewal of postnatal neural stem cells[45,47], conditional loss-of-function studies should provide more direct evidence for the role of Bmi1 in regulating adult neural stem cells in the SVZ. It also remains mechanistically unclear how Bmi1 would maintain the self-renewal of adult neural stem cells in the SVZ by regulating methylation of its target H3K27. One appealing model is that the methylation of H3K27 by the PcG complex silences crucial genes that are required for neural differentiation over many cell divisions to promote self-renewal as a form of 'cellular memory'[43,44].

Methyl-CpG binding protein 1 (Mbd1) has emerged as a crucial and specific regulator of adult neural stem cells in the SGZ. Mbd1 knockout mice show no detectable developmental defects and appear healthy throughout life, but they have severely reduced adult neurogenesis and impaired spatial learning[48]. Among several Mbd family proteins, only Mbd1 specifically affects postnatal neurogenesis in the SGZ[48,49]. Mechanistically, Mbd1 binds to the promoter of the gene that encodes fibroblast growth factor 2 (Fgf2), a mitogen for adult neural progenitors, and regulates its expression in adult SGZ neural progenitors in a manner that depends on DNA methylation[48,50]. The regulated expression of Fgf2 in adult neural stem cells might allow temporally appropriate neuronal differentiation both *in vitro* and *in vivo*. Surprisingly, as a methyl-CpG 'reader' protein, Mbd1 also seems to affect DNA methylation levels *per se*[50], suggesting that Mbd1 may recruit unidentified DNA methyltransferases to form propagating feedback loops that silence its target genes over many rounds of cell division. Such long-term silencing propagation would, in principle, be similar to the action of the PcG complex in promoting the self-renewal of adult neural stem cells in the SVZ, although these two mechanisms enact different epigenetic silencing machineries in different brain regions.

In addition to PcG-mediated histone modifications and DNA methylation, self-sustaining feedback loops that are mediated by transcription factors are truly epigenetic in nature[29] and have critical roles in maintaining adult neural stem cells. A member of the Sox (SRY-related HMG box) transcription factor family, Sox2 is highly expressed in both SVZ and SGZ neural stem cells but not in their neuronal progeny[51,52]. Sox2 gene products bind to numerous genomic targets, including its own promoter, to form a feedback loop, which is characteristic of the cell regulatory circuitry for self-renewing stem cells[51,53,54]. Together with several other transcription factors, Sox2 might constitute a key part of the feedback and feedforward networks in regulating other downstream genes that are crucial for stem cell maintenance. In adult mice, Sox2 marks a self-renewing, multipotent population of SGZ neural stem cells and is required for their self-renewal and maintenance of neuronal developmental potential[52]. In addition to autoregulatory loops, Sox2 participates in another feedback pathway by enhancing the expression of epidermal growth factor receptor (Egfr), which, in turn, upregulates Sox2 expression in neural stem cells[55]. Sox2 might thus promote self-renewal and mediate epigenetic inheritance of adult neural stem cell properties by forming self-sustaining transcriptional pathways that are immune to the perturbations of cell divisions but remain permissive to multi-lineage differentiation cues.

Epigenetic regulation of neural stem cell differentiation

Unlike the silencing PcG complex, TrxG proteins establish stable and transcriptionally active chromatin domains by catalyzing and

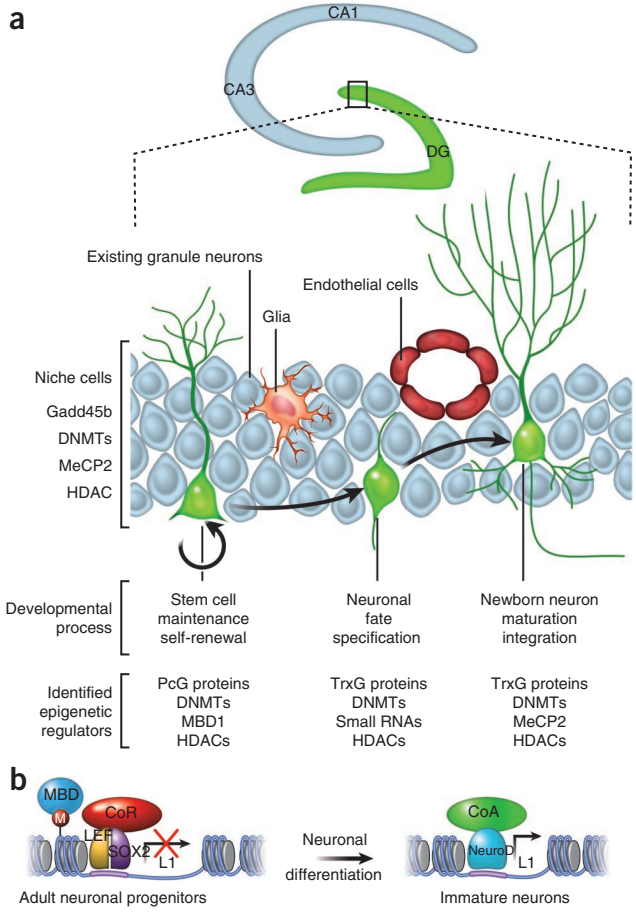

Figure 3 Major epigenetic regulators of adult neurogenesis. (**a**) Current understanding of epigenetic regulation of adult neurogenesis in SGZ and SVZ. Adult neural progenitors undergo proliferation and generate neuroblasts that can further differentiate into mature, functional neurons. Neurogenesis can be regulated by intrinsic epigenetic mechanisms within the neuronal lineage of adult neural progenitors and extrinsically by nearby niche signaling cells, such as mature neurons, astrocytes and endothelial cells. During the stages of adult neurogenesis, diverse epigenetic mechanisms use common or different sets of molecules, including DNMT, PcG and TrxG, HDAC, MBD and Gadd45 family proteins, in either adult neural progenitors and their progeny or nearby niche cells to choreograph cell state transitions in coordination with other internal and external cues. Common molecules, such as HDACs, are likely to have different partners or binding sites depending on the differentiation stage during neurogenesis. Prototypical cell stages are shown to reflect identified epigenetic regulators for both SVZ and SGZ adult neurogenesis. (**b**) During early stages of adult hippocampal neurogenesis in the SGZ, L1 transcription is regulated by the transcription factors Sox2 and TCF to maintain the long-term silencing state, even during cell division. In response to external stimuli such as neuronal activity or Wnt signaling, the balance of co-repressor and co-activator in the L1 promoter is tipped to activation of L1 expression, driving its genomic retrotransposition. After terminal differentiation, adult neural progenitor–specific expression of Sox2 is downregulated and L1 transcription and retrotransposition are decreased. DNA methylation and MBD-mediated epigenetic mechanisms could act to ensure the long-term silencing of L1 by recruiting transcriptional co-repressors. CoA, co-activator; CoR, co-repressor; DG, dentate gyrus.

maintaining histone tail H3K4 methylation[43]. One TrxG member, Mll1 (mixed-lineage leukemia 1), encodes an H3K4 methyltransferase. Mll1 is specifically required for neuronal, but not glial, differentiation from adult neural stem cells[56]. The homeobox protein Dlx2 has been identified as one direct target of Mll1 and is crucial for neurogenesis in the SVZ. ChIP analysis has provided evidence that Mll1 binds to the Dlx2 promoter, but H3K4 methylation is normal in the Mll1 binding loci of Dlx2 in differentiating SVZ cells from mice lacking Mll1. Instead, Mll1 deficiency leads to the abnormal presence of a bivalently marked chromatin, with both H3K4 and H3K27 methylation, at the Dlx2 locus. Mll1 therefore seems to recruit an H3K27 demethylase that is required to resolve silenced bivalent loci in SVZ neural stem cells for proper neuronal, but not glial, differentiation. More recent work suggests that Jmjd3, an H3K27 demethylase, is required for neuronal fate commitment and terminal differentiation from neural stem cells during embryonic development[57]. It is unclear whether such a TrxG-mediated chromatin switching mechanism might directly oppose the PcG-mediated cellular silencing program. The H3K27 demethylase that is requited for neurogenesis in the adult SVZ also remains to be identified. Nonetheless, current evidence suggests that the TrxG complex cooperates with PcG antagonists to help to switch neural stem cells from a PcG-mediated self-renewal state into a neuronal differentiation state.

Histone acetylation and deacetylation are also powerful epigenetic mechanisms that are associated with gene activation or repression, respectively, through histone acetyltransfeases (HATs) and deacetylases (HDACs; **Fig. 1c**). Inactivation of HDAC activities by HDAC inhibitors leads to marked enhancement of neuronal differentiation from adult neural stem cells in the SGZ[58]. In these stem cells, HDAC silences the expression of key neurogenic transcription factors, such as NeuroD1, and of cell-cycle regulators through gene-specific recruitment by the transcription factor Tlx1 (ref. 59). A deficiency of one member of the HDAC family, HDAC2, results in specific and cell-autonomous defects in neural differentiation during adult but not embryonic neurogenesis[60]. Compared with PcG-mediated silencing mechanisms, which are stable, HDAC-mediated histone deacetylation seems to be more dynamic[61–63]. The use of epigenetic marks with distinct turnover properties might enable adult neural stem cells to control different complements of genes that are required for appropriately timed cell-cycle exit, fate specification and terminal differentiation programs (**Fig. 3a**).

Small non-coding RNAs (microRNAs or miRNAs) have emerged as an important class of gene regulators that use different mechanisms of action but form intricate regulatory interaction networks with chromatin modifiers and transcription factors[64–66]. Many members of the small RNA family that regulate adult neurogenesis have been identified (**Fig. 3a**). First, miR-184 functions as a direct target of Mbd1 to inhibit neuronal differentiation from adult neural stem cells in the SGZ[67]. Mechanistically, miR-184 acts through post-transcriptional repression of Numb-like, which regulates neuronal differentiation during development. Second, miR-137 has been identified as a direct target of Sox2 and another DNA methyl-CpG-binding protein, MeCP2, that inhibits neuronal differentiation and maturation in adult SGZ neural stem cells[68,69]. Interestingly, miR-137 represses the translation of Ezh2, an H3K27 methyltransferase and PcG complex protein, and this leads to a global reduction of H3K27 methylation in adult neural stem cells in the SGZ[68]. Thus, adult SGZ neural stem cells may require miR-137 to balance global H3K27 levels while maintaining gene-specific bivalent domains through PcG-mediated epigenetic mechanisms. Third, one of the most abundant microRNAs in the adult brain, miR-124, is both required and sufficient to promote neuronal differentiation from adult SVZ neural stem cells[70]. Interestingly, one crucial target that mediates the action of miR-124 in this system is another Sox family protein, Sox9. Functionally, miR-124–mediated repression of Sox9 ensures correct cell state progression along the SVZ stem cell lineage to neurons. In addition, a

small modulatory RNA has also been shown to trigger neuronal gene expression from adult neural stem cells by inhibiting the action of the REST-NRSF (repressor element-1 silencing transcription factor and neuron restrictive silencer factor) transcriptional machineries[71]. REST-NRSF potently represses neuronal genes, partly by suppressing miR-124 and switching the ATP-dependent chromatin remodeling complexes during neural differentiation[72,73]. There is therefore accumulating evidence that diverse members of the small RNA family are recruited for fine-tuning cellular epigenetic programs to ensure temporally choreographed transitions between cell differentiation states during adult neurogenesis.

Long interspersed nuclear element-1 (L1) comprises about 17% of the human genome. It is thought to be active only in the germline or during early development and is silenced by epigenetic modifications such as DNA methylation during somatic cell differentiation (**Fig. 3b**). However, recent studies using both rodent and human neural progenitors have provided surprising evidence that L1 can be actively retrotransposing and thus modifying the genomic sequence during neuronal differentiation[74,75]. Such non-epigenetic mechanisms might help to create neuronal diversity by fine-tuning the expression of neuronal genes on a cell-by-cell basis and could also contribute to individual somatic mosaicism in the adult brain[76]. Even though post-insertional mutagenesis of L1 in neurons is not strictly considered to be epigenetic, the expression of L1 before insertion during neurogenesis is believed to be controlled by epigenetic mechanisms. In humans, L1 seems to be more active in the brain than in other tissues and its activity is inversely correlated with the DNA methylation of its regulatory sites from different tissues[74]. In adult neural stem cells, the transcription factor Sox2 (ref. 75) and the Wnt signaling effector Tcf[77] associate with the L1 promoter and coordinate to regulate L1 transcription[75]. The simultaneous presence of both activating transcription factors and repressing silencing effectors may be important to maintain the L1 promoter in a poised state. Interestingly, both voluntary exercise and extracellular Wnt signaling seem to activate L1 retrotranspositions[77,78]. Furthermore, L1 retrotranspositions can alter neuronal gene expression to functionally affect neuronal differentiation processes[75]. Epigenetic processes that tightly regulate L1 activity might therefore represent another intriguing cellular response mechanism to external cues that could modulate adult hippocampal neurogenesis by mobilizing DNA retrotransposons.

Epigenetic regulation of the neurogenic niche

The entire process of adult neurogenesis is dynamically modulated by many environmental stimuli, local niche signals and diverse external cues[4,21,79]. Thus, epigenetic mechanisms involved in adult neurogenesis also include those that take place in adult neurogenic niche cells that actively communicate with and regulate the development of adult neural stem cells and their progeny. Although the epigenetic machinery in adult neural stem cells and their progeny may respond directly to ambient signals, there is emerging evidence that specialized niche cells, such as mature neurons, are ideally suited to use their own epigenetic mechanisms to translate various transient external cues into long-lasting modifications of adult neurogenic processes.

Gadd45b has been identified as a key epigenetic regulator that links activity-induced changes in DNA methylation in mature neurons to modulation of neurogenesis in the adult SGZ[80,81]. Gadd45b is an activity-induced immediate early gene, so its transcription is sensitive to various transient stimuli that can stably increase adult neurogenesis[80]. Activity-induced expression of Gadd45b in mature dentate granule neurons near the SGZ promotes DNA demethylation of several genes that are crucial for adult neurogenesis, including the genes for brain-derived neurotrophic factor (Bdnf) and fibroblast growth factor (Fgf), with demethylation occurring in their specific promoters. After transient stimulation, the demethylated status of CpGs within these promoters persists for at least several days. Furthermore, Gadd45b knockout mice show specific deficits in neuronal activity-induced proliferation of adult neural progenitors and dendritic growth of newborn neurons. Thus, Gadd45b seems to function as a sensor in mature neurons for environmental stimuli and translates transient neural activity into relatively more stable nuclear epigenetic changes in DNA methylation to elicit lasting modifications of local circuit connectivity through adult neurogenesis.

In mature neurons, MeCP2 is another key epigenetic regulator that directly controls Bdnf expression in an activity-dependent manner[82–84]. MeCP2 is abundant in mature dentate granule neurons, and MeCP2 knockout mice show pronounced deficits in the maturation of newborn neurons in the SGZ, including delayed neuronal differentiation, altered neuronal gene expression and reduced dendritic spine density[85]. It is unclear to what extent the deficiency of MeCP2 contributes to these neuronal maturation defects in mature neurons and glia as niche cells or in newborn neurons in a cell-autonomous fashion. One role for MeCP2 in mature neurons might be to interpret the reversible DNA methylation status of genes mediated by activity-regulated actions of Dnmts and Gadd45b proteins[80,86]. As well as DNA methylation, other epigenetic modifications seem to be dynamically controlled by the activity-dependent regulation of chromatin remodeling and modifying enzymes, including HATs and HDACs in mature neurons[61,62,87]. Regulation of niche signaling molecules such as Bdnf by these mechanisms in mature dentate neurons might serve as an additional mechanism through which epigenetic processes influence neurogenesis.

Endothelial and glial cells function in both the SVZ and the SGZ as important niche cells for adult neurogenesis[79]. Niche signaling molecules have been shown to regulate various process in adult neurogenesis[5,88]. Therefore, epigenetic analyses of these cells under defined stimulus conditions represent interesting avenues for future exploration.

Implications for brain tumors and mental disorders

Adult neurogenesis is a highly orchestrated process that requires extensive epigenetic mechanisms for its regulation. Conversely, epigenetic dysregulation of adult neurogenesis can lead to pathological cell states, as seen in many neurological diseases. The key regulator of self-renewal for adult neural stem cells in the SVZ, Bmi1, is an oncogene; its overexpression or overactivation of the associated PcG complex stimulates tumor growth in several types of glioblastoma and specifically sustains the self-renewal of cancer stem cells[42,89]. Similarly, many types of brain tunor that are resistant to cell differentiation, which might originate from adult neural progenitors, have abnormal patterns of DNA methylation and histone modification, reflecting the failure of epigenetic switches that normally guide them into neural differentiation pathways[89,90].

A wide variety of detrimental stress stimuli that can lead to depression generally reduce the rate of adult SGZ neurogenesis, whereas chemical antidepressants and electroconvulsive treatments (ECT) markedly enhance neurogenesis in the adult SGZ[91,92]. Functionally, adult SGZ neurogenesis has been suggested to mediate the effects of antidepressants[92]. The finding that Gadd45b is strongly activated by ECT and is required for ECT-induced adult neurogenesis suggests that it might help to mediate the effects of antidepressants and also implicates epigenetic mechanisms involving changes in DNA methylation in the pathological processes that lead to depression.

Several neuropsychiatric disorders in humans or animal models feature defective or abnormal adult neurogenesis caused by aberrant epigenetic mechanisms that regulate intrinsic or extrinsic aspects of adult neurogenesis[5,19,21]. Mouse models of the autistic spectrum disorder Rett syndrome, in which the disease is caused by a deficiency in MeCP2, show maturation defects in adult-born neurons. Several core deficits that are frequently associated with autism are seen in Mbd1 knockout mice, which have markedly reduced adult SGZ neurogenesis and dysregulation of adult neural progenitors in the SGZ[93]. Bdnf, a regulator of adult neurogenesis, is subjected to diverse epigenetic regulation[80,94–97]. The human variant of Bdnf that is crucial genetic predispositions to depressive disorders causes anxiety-related behaviors when modeled in mice and impairs neurogenesis in the adult SVZ and olfactory behavior[98,99]. In many cases, it remains to be investigated whether specific disease-related gene deficiencies can cell-autonomously cause the observed phenotypes and how abnormal adult neurogenesis might directly contribute to disease etiology and manifestation of symptoms. Nonetheless, adult neurogenesis is highly sensitive to many physiological, pathological and environmental stimuli, responding with diverse epigenetic mechanisms for its intrinsic and extrinsic regulation. Therefore, we propose that studies of explicitly observable and explicable phenotypic defects in any aspect of the process of adult neurogenesis will provide a unique glimpse into the pathology and mechanistic basis of brain disorders[100].

Conclusions

Adult neurogenesis is a developmental process that occurs in the environment of the mature nervous system and represents a striking form of neural plasticity in the adult brain. The process of adult neurogenesis is intrinsically controlled by extensive epigenetic mechanisms, including DNA methylation, histone modification and transcriptional feedback loops, which ensure that cells undergo transitions through sequential stages of neurogenesis in coordination with other cellular programs. Long-lasting effects elicited by some of these epigenetic mechanisms seem to mainly promote the self-renewal of adult neural stem cells. Bridging the gap between the environment and the genome, epigenetic mechanisms also act extrinsically through neurogenic niche cells, translating transient environmental signals into relatively long-term modification of neurogenesis in the adult brain. Together, intrinsic and extrinsic epigenetic mechanisms help to maintain adult neurogenesis throughout life and contribute to its activity-dependent regulation.

Epigenetics, especially in the field of adult neurogenesis, is still in its nascent stage. Technical challenges have largely limited the widespread application of the conventional epigenetic toolkit for studying adult neurogenesis *in vivo*. Several emerging technologies are now overcoming technical obstacles to enable epigenetic analysis to be carried out in a more accurate and sensitive manner and on a genome-wide scale. The next few years will probably see exciting progress toward answering current questions and the discovery of new modes and principles that underlie the epigenetic regulation of adult neurogenesis. Recapitulating almost the entire neuronal developmental process—including neural fate specification, neuronal morphogenesis, migration, axonal and dendritic development, and the formation, maturation and maintenance of synapses—adult neurogenesis also presents an excellent model for understanding the biological roles of many disease-susceptibility genes that are involved in epigenetic processes that regulate specific aspects of neural development and functions. Therefore, future research in the field should greatly facilitate our understanding of those diseases and provide potential new avenues for therapeutic development.

ACKNOWLEDGMENTS

We thank M.L. Gage and K. Christian for comments on the manuscript and J. Simon for illustrations. This work was supported by the National Institutes of Health grants NS048271 and HD069184 (G.-l.M.), MH088485 and MH090258 (F.H.G.), and AG024984, NS047344 and MH087874 (H.S.), the Brain Science Institute at Johns Hopkins and National Alliance for Research on Schizophrenia and Depression (G.-l.M.), the Mathers Foundation, the Lookout Fund and the J.S. McDonnell Foundation (F.H.G.), a Helen Hay Whitney Foundation postdoctoral fellowship (D.K.M.), and the Dr. Richard and Mavis Fowler and the Foundation for Advanced Research in the Medical Sciences Fellowship (J.U.G.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/natureneuroscience/.
Reprints and permissions information is available online at http://www.nature.com/reprintsandpermissions/.

1. Cameron, H.A. & McKay, R.D. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J. Comp. Neurol.* **435**, 406–417 (2001).
2. Altman, J. & Das, G.D. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* **124**, 319–335 (1965).
3. Gage, F.H. Mammalian neural stem cells. *Science* **287**, 1433–1438 (2000).
4. Alvarez-Buylla, A. & Lim, D.A. For the long run: maintaining germinal niches in the adult brain. *Neuron* **41**, 683–686 (2004).
5. Ming, G.L. & Song, H. Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223–250 (2005).
6. Lledo, P.M., Alonso, M. & Grubb, M.S. Adult neurogenesis and functional plasticity in neuronal circuits. *Nat. Rev. Neurosci.* **7**, 179–193 (2006).
7. Zhao, C., Deng, W. & Gage, F.H. Mechanisms and functional implications of adult neurogenesis. *Cell* **132**, 645–660 (2008).
8. Ma, D.K., Bonaguidi, M.A., Ming, G.L. & Song, H. Adult neural stem cells in the mammalian central nervous system. *Cell Res.* **19**, 672–682 (2009).
9. Duan, X., Kang, E., Liu, C.Y., Ming, G.L. & Song, H. Development of neural stem cell in the adult brain. *Curr. Opin. Neurobiol.* **18**, 108–115 (2008).
10. Kempermann, G., Wiskott, L. & Gage, F.H. Functional significance of adult neurogenesis. *Curr. Opin. Neurobiol.* **14**, 186–191 (2004).
11. Deng, W., Aimone, J.B. & Gage, F.H. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* **11**, 339–350 (2010).
12. Kitabatake, Y., Sailor, K.A., Ming, G.L. & Song, H. Adult neurogenesis and hippocampal memory function: new cells, more plasticity, new memories? *Neurosurg. Clin. N. Am.* **18**, 105–113 (2007).
13. Holliday, R. Mechanisms for the control of gene activity during development. *Biol. Rev. Camb. Philos. Soc.* **65**, 431–471 (1990).
14. Goldberg, A.D., Allis, C.D. & Bernstein, E. Epigenetics: a landscape takes shape. *Cell* **128**, 635–638 (2007).
15. Waddington, C.H. *Principles of Development and Differentiation* (Macmillan, New York, 1966).
16. Holliday, R. Epigenetics: a historical overview. *Epigenetics* **1**, 76–80 (2006).
17. Jaenisch, R. & Bird, A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* **33** Suppl., 245–254 (2003).
18. Zhang, T.Y. & Meaney, M.J. Epigenetics and the environmental regulation of the genome and its function. *Annu. Rev. Psychol.* **61**, 439–466 (2010).
19. Covic, M., Karaca, E. & Lie, D.C. Epigenetic regulation of neurogenesis in the adult hippocampus. *Heredity* **105**, 122–134 (2010).
20. Hsieh, J. & Eisch, A.J. Epigenetics, hippocampal neurogenesis and neuropsychiatric disorders: unraveling the genome to understand the mind. *Neurobiol. Dis.* **39**, 73–84 (2010).
21. Ma, D.K., Kim, W.R., Ming, G.L. & Song, H. Activity-dependent extrinsic regulation of adult olfactory bulb and hippocampal neurogenesis. *Ann. NY Acad. Sci.* **1170**, 664–673 (2009).
22. Cedar, H. & Bergman, Y. Linking DNA methylation and histone modification: patterns and paradigms. *Nat. Rev. Genet.* **10**, 295–304 (2009).
23. Holliday, R. Epigenetic inheritance based on DNA methylation. *EXS* **64**, 452–468 (1993).
24. Kriaucionis, S. & Heintz, N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* **324**, 929–930 (2009).
25. Tahiliani, M. *et al.* Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324**, 930–935 (2009).
26. Ito, S. *et al.* Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* **466**, 1129–1133 (2010).
27. Jenuwein, T. & Allis, C.D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
28. Serio, T.R. & Lindquist, S.L. Protein-only inheritance in yeast: something to get [PSI+]-ched about. *Trends Cell Biol.* **10**, 98–105 (2000).
29. Ptashne, M. On the use of the word 'epigenetic'. *Curr. Biol.* **17**, R233–R236 (2007).
30. Capela, A. & Temple, S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron* **35**, 865–875 (2002).

31. Pastrana, E., Cheng, L.C. & Doetsch, F. Simultaneous prospective purification of adult subventricular zone neural stem cells and their progeny. *Proc. Natl. Acad. Sci. USA* **106**, 6387–6392 (2009).
32. Kanki, H., Shimabukuro, M.K., Miyawaki, A. & Okano, H. "Color Timer" mice: visualization of neuronal differentiation with fluorescent proteins. *Mol. Brain* **2**, 3 (2010).
33. Encinas, J.M. & Enikolopov, G. Identifying and quantitating neural stem and progenitor cells in the adult brain. *Methods Cell Biol.* **85**, 243–272 (2008).
34. Heiman, M. *et al.* A translational profiling approach for the molecular characterization of CNS cell types. *Cell* **135**, 738–748 (2008).
35. Goren, A. *et al.* Chromatin profiling by directly sequencing small quantities of immunoprecipitated DNA. *Nat. Methods* **7**, 47–49 (2010).
36. O'Neill, L.P., VerMilyea, M.D. & Turner, B.M. Epigenetic characterization of the early embryo with a chromatin immunoprecipitation protocol applicable to small cell populations. *Nat. Genet.* **38**, 835–841 (2006).
37. Ball, M.P. *et al.* Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat. Biotechnol.* **27**, 361–368 (2009).
38. Lister, R. *et al.* Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462**, 315–322 (2009).
39. Bernstein, B.E. *et al.* A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315–326 (2006).
40. Roh, T.Y., Ngau, W.C., Cui, K., Landsman, D. & Zhao, K. High-resolution genome-wide mapping of histone modifications. *Nat. Biotechnol.* **22**, 1013–1016 (2004).
41. Jin, S.G., Kadam, S. & Pfeifer, G.P. Examination of the specificity of DNA methylation profiling techniques towards 5-methylcytosine and 5-hydroxymethylcytosine. *Nucleic Acids Res.* **38**, e125 (2010).
42. Molofsky, A.V. *et al.* Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* **425**, 962–967 (2003).
43. Ringrose, L. & Paro, R. Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development* **134**, 223–232 (2007).
44. Ng, R.K. & Gurdon, J.B. Epigenetic inheritance of cell differentiation status. *Cell Cycle* **7**, 1173–1177 (2008).
45. Fasano, C.A. *et al.* Bmi-1 cooperates with Foxg1 to maintain neural stem cell self-renewal in the forebrain. *Genes Dev.* **23**, 561–574 (2009).
46. Fasano, C.A. *et al.* shRNA knockdown of Bmi-1 reveals a critical role for p21-Rb pathway in NSC self-renewal during development. *Cell Stem Cell* **1**, 87–99 (2007).
47. He, S. *et al.* Bmi-1 over-expression in neural stem/progenitor cells increases proliferation and neurogenesis in culture but has little effect on these functions in vivo. *Dev. Biol.* **328**, 257–272 (2009).
48. Zhao, X. *et al.* Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. *Proc. Natl. Acad. Sci. USA* **100**, 6777–6782 (2003).
49. Martín Caballero, I., Hansen, J., Leaford, D., Pollard, S. & Hendrich, B.D. The methyl-CpG binding proteins Mecp2, Mbd2 and Kaiso are dispensable for mouse embryogenesis, but play a redundant function in neural differentiation. *PLoS ONE* **4**, e4315 (2009).
50. Li, X. *et al.* Epigenetic regulation of the stem cell mitogen Fgf-2 by Mbd1 in adult neural stem/progenitor cells. *J. Biol. Chem.* **283**, 27644–27652 (2008).
51. Episkopou, V. SOX2 functions in adult neural stem cells. *Trends Neurosci.* **28**, 219–221 (2005).
52. Suh, H. *et al.* In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippoampus. *Cell Stem Cell* **1**, 515–528 (2007).
53. Boyer, L.A. *et al.* Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947–956 (2005).
54. Jaenisch, R. & Young, R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* **132**, 567–582 (2008).
55. Hu, Q. *et al.* The EGF receptor-sox2-EGF receptor feedback loop positively regulates the self-renewal of neural precursor cells. *Stem Cells* **28**, 279–286 (2010).
56. Lim, D.A. *et al.* Chromatin remodeling factor Mll1 is essential for neurogenesis from postnatal neural stem cells. *Nature* **458**, 529–533 (2009).
57. Jepsen, K. *et al.* SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. *Nature* **450**, 415–419 (2007).
58. Hsieh, J., Nakashima, K., Kuwabara, T., Mejia, E. & Gage, F.H. Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. *Proc. Natl. Acad. Sci. USA* **101**, 16659–16664 (2004).
59. Sun, G., Yu, R.T., Evans, R.M. & Shi, Y. Orphan nuclear receptor TLX recruits histone deacetylases to repress transcription and regulate neural stem cell proliferation. *Proc. Natl. Acad. Sci. USA* **104**, 15282–15287 (2007).
60. Jawerka, M. *et al.* The specific role of histone deacetylase 2 in adult neurogenesis. *Neuron Glia Biol.* published online, doi:10.1017/S1740925X10000049 (14 April 2010).
61. Crepaldi, L. & Riccio, A. Chromatin learns to behave. *Epigenetics* **4**, 23–26 (2009).
62. Zocchi, L. & Sassone-Corsi, P. Joining the dots: from chromatin remodeling to neuronal plasticity. *Curr. Opin. Neurobiol.* **20**, 432–440 (2010).
63. Wang, Z. *et al.* Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* **138**, 1019–1031 (2009).
64. Morris, K.V. Non-coding RNAs, epigenetic memory and the passage of information to progeny. *RNA Biol.* **6**, 242–247 (2009).
65. Hobert, O. Gene regulation by transcription factors and microRNAs. *Science* **319**, 1785–1786 (2008).
66. Iorio, M.V., Piovan, C. & Croce, C.M. Interplay between microRNAs and the epigenetic machinery: an intricate network. *Biochim. Biophys. Acta* published online, doi:10.1016/j.bbagrm.2010.05.005 (20 May 2010).
67. Liu, C. *et al.* Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation. *Cell Stem Cell* **6**, 433–444 (2010).
68. Szulwach, K.E. *et al.* Cross talk between microRNA and epigenetic regulation in adult neurogenesis. *J. Cell Biol.* **189**, 127–141 (2010).
69. Smrt, R.D. *et al.* MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. *Stem Cells* **28**, 1060–1070 (2010).
70. Cheng, L.C., Pastrana, E., Tavazoie, M. & Doetsch, F. miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat. Neurosci.* **12**, 399–408 (2009).
71. Kuwabara, T., Hsieh, J., Nakashima, K., Taira, K. & Gage, F.H. A small modulatory dsRNA specifies the fate of adult neural stem cells. *Cell* **116**, 779–793 (2004).
72. Juliandi, B., Abematsu, M. & Nakashima, K. Chromatin remodeling in neural stem cell differentiation. *Curr. Opin. Neurobiol.* **20**, 408–415 (2010).
73. Yoo, A.S., Staahl, B.T., Chen, L. & Crabtree, G.R. MicroRNA-mediated switching of chromatin-remodelling complexes in neural development. *Nature* **460**, 642–646 (2009).
74. Coufal, N.G. *et al.* L1 retrotransposition in human neural progenitor cells. *Nature* **460**, 1127–1131 (2009).
75. Muotri, A.R. *et al.* Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. *Nature* **435**, 903–910 (2005).
76. Muotri, A.R. & Gage, F.H. Generation of neuronal variability and complexity. *Nature* **441**, 1087–1093 (2006).
77. Kuwabara, T. *et al.* Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. *Nat. Neurosci.* **12**, 1097–1105 (2009).
78. Muotri, A.R., Zhao, C., Marchetto, M.C. & Gage, F.H. Environmental influence on L1 retrotransposons in the adult hippocampus. *Hippocampus* **19**, 1002–1007 (2009).
79. Ma, D.K., Ming, G.L. & Song, H. Glial influences on neural stem cell development: cellular niches for adult neurogenesis. *Curr. Opin. Neurobiol.* **15**, 514–520 (2005).
80. Ma, D.K. *et al.* Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* **323**, 1074–1077 (2009).
81. Ma, D.K., Guo, J.U., Ming, G.L. & Song, H. DNA excision repair proteins and Gadd45 as molecular players for active DNA demethylation. *Cell Cycle* **8**, 1526–1531 (2009).
82. Martinowich, K. *et al.* DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* **302**, 890–893 (2003).
83. Zhou, Z. *et al.* Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth and spine maturation. *Neuron* **52**, 255–269 (2006).
84. He, D.Y., Neasta, J. & Ron, D. Epigenetic regulation of BDNF expression via the scaffolding protein rack1. *J. Biol. Chem.* **285**, 19043–19050 (2010).
85. Smrt, R.D. *et al.* Mecp2 deficiency leads to delayed maturation and altered gene expression in hippocampal neurons. *Neurobiol. Dis.* **27**, 77–89 (2007).
86. Feng, J. *et al.* Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat. Neurosci.* **13**, 423–430 (2010).
87. Qiu, Z. & Ghosh, A. A calcium-dependent switch in a CREST-BRG1 complex regulates activity-dependent gene expression. *Neuron* **60**, 775–787 (2008).
88. Mu, Y., Lee, S.W. & Gage, F.H. Signaling in adult neurogenesis. *Curr. Opin. Neurobiol.* **20**, 416–423 (2010).
89. Dirks, P. Bmi1 and cell of origin determinants of brain tumor phenotype. *Cancer Cell* **12**, 295–297 (2007).
90. Nagarajan, R.P. & Costello, J.F. Epigenetic mechanisms in glioblastoma multiforme. *Semin. Cancer Biol.* **19**, 188–197 (2009).
91. Banasr, M. & Duman, R.S. Regulation of neurogenesis and gliogenesis by stress and antidepressant treatment. *CNS Neurol. Disord. Drug Targets* **6**, 311–320 (2007).
92. Sahay, A. & Hen, R. Adult hippocampal neurogenesis in depression. *Nat. Neurosci.* **10**, 1110–1115 (2007).
93. Allan, A.M. *et al.* The loss of methyl-CpG binding protein 1 leads to autism-like behavioral deficits. *Hum. Mol. Genet.* **17**, 2047–2057 (2008).
94. Li, Y. *et al.* TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron* **59**, 399–412 (2008).
95. Roth, T.L. & Sweatt, J.D. Epigenetic marking of the BDNF gene by early-life adverse experiences. *Horm. Behav.* published online, doi:10.1016/j.yhbeh.2010.05.005 (17 May 2010).
96. Kuzumaki, N. *et al.* Hippocampal epigenetic modification at the brain-derived neurotrophic factor gene induced by an enriched environment. *Hippocampus* published online, doi:10.1002/hipo.20775 (15 March 2010).
97. Nelson, E.D., Kavalali, E.T. & Monteggia, L.M. Activity-dependent suppression of miniature neurotransmission through the regulation of DNA methylation. *J. Neurosci.* **28**, 395–406 (2008).
98. Bath, K.G. *et al.* Variant brain-derived neurotrophic factor (Val66Met) alters adult olfactory bulb neurogenesis and spontaneous olfactory discrimination. *J. Neurosci.* **28**, 2383–2393 (2008).
99. Chen, Z.Y. *et al.* Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* **314**, 140–143 (2006).
100. Christian, K., Song, H. & Ming, G.L. Adult neurogenesis as a cellular model to study schizophrenia. *Cell Cycle* **9**, 636–637 (2010).