# Epigenetic mechanisms in neurogenesis

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Abstract | In the embryonic and adult brain, neural stem cells proliferate and give rise to neurons and glia through highly regulated processes. Epigenetic mechanisms — including DNA and histone modifications, as well as regulation by non-coding RNAs — have pivotal roles in different stages of neurogenesis. Aberrant epigenetic regulation also contributes to the pathogenesis of various brain disorders. Here, we review recent advances in our understanding of epigenetic regulation in neurogenesis and its dysregulation in brain disorders, including discussion of newly identified DNA cytosine modifications. We also briefly cover the emerging field of epitranscriptomics, which involves modifications of mRNAs and long non-coding RNAs.

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The concept of epigenetics was first introduced almost a century ago to describe the molecular events that are involved in early embryonic development[1]. Epigenetics is now widely accepted as the study of changes in gene expression that do not result from alteration in DNA sequence[2]. Several classic epigenetic mechanisms have been extensively investigated, including DNA methylation, histone modifications, chromatin remodelling and regulation mediated by non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs)[2] (BOX 1). More recently, novel DNA and RNA chemical modifications have been investigated, many of which are enriched in the mammalian CNS[3,4]. Epigenetic modifications can be dynamically regulated by sets of enzymes that serve as 'writers' or 'erasers' to add or remove specific epigenetic marks, respectively, and by 'readers' that bind to these modifications and serve as effectors.

Neurogenesis is the process through which neural stem cells (NSCs), or more generally neural progenitor cells (NPCs), generate new neurons[5,6]. This process occurs not only during embryonic and perinatal stages but also throughout life in two discrete regions of the mammalian CNS: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus[7]. Adult neurogenesis can also occur to a much lesser degree in non-canonical sites, under both basal conditions and in response to injury[8]. Multiple epigenetic mechanisms orchestrate neurogenesis through coordinated responses to extracellular cues, which determine the spatial and temporal expression of key regulators that control the proliferation, fate specification and differentiation of NPCs[9,10]. Here, we review recent progress in our understanding of the epigenetic mechanisms that regulate neurogenesis, with a focus on dynamic DNA and histone modifications.

## Embryonic and adult neurogenesis

Embryonic neurogenesis in the mouse brain begins with the transformation of neuroepithelial cells that are located in the ventricular zone (VZ) and SVZ into radial glial cells (RGCs)[11] (FIG. 1a). RGCs initially function as fate-restricted NPCs that either directly generate nascent neurons or produce neuronal intermediate progenitor cells (IPCs), which in turn give rise to neurons through symmetrical mitosis[11]. As neuroepithelial cells transform into RGCs, they start to lose certain epithelial features, such as tight junctions, and acquire astroglial properties, including the expression of several astrocytic markers[12,13]. This transition occurs in a relatively narrow time window in rodents. There is no detectable expression of astroglial markers in cells of embryonic day 10 (E10) mice, but these markers can be clearly detected at E12 (REFS 14,15). Many intrinsic signals, including rapid epigenetic changes, work synergistically to support this transition and ensure robust embryonic neurogenesis[11,16–18]. Later in development, RGCs also participate in the production of astrocytes and oligodendrocytes. Although the majority of RGCs terminally differentiate into neural cells by the end of development, a small population of RGCs remains quiescent during the embryonic stage; these residual cells become the stem cells that are responsible for adult SVZ neurogenesis[19,20].

In the adult SVZ, these quiescent radial glia-like neural stem cells (RGLs) can be activated and give rise to IPCs, which in turn produce neuroblasts[6] (FIG. 1b). Neuroblasts and their immature neuronal progeny travel in chains through the rostral migratory stream to the olfactory bulb, where they differentiate into interneurons. Adult SVZ RGLs are also known to give rise to oligodendrocytes[7]. In the SGZ of the adult mouse hippocampus, RGLs produce T-box brain protein 2 (TBR2)-expressing IPCs that give rise to neuroblasts, which in turn differentiate into dentate

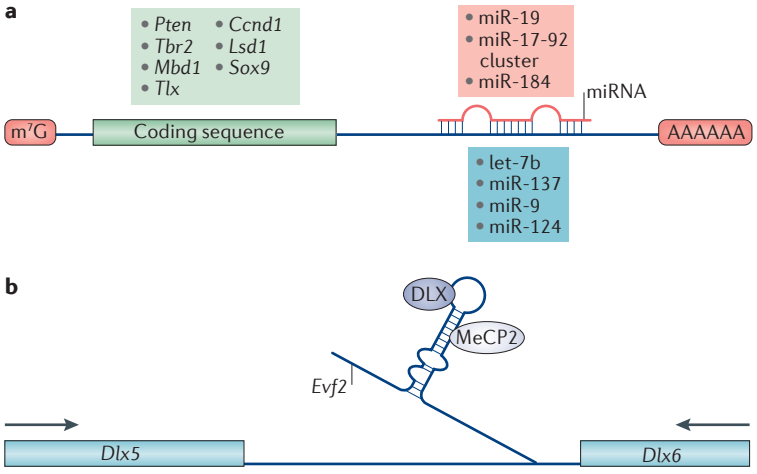
Neural progenitor cells (NPCs). Precursor cells of the nervous system that can produce more of themselves and differentiate into various types of neural cells.

granule neurons that are distributed locally in the dentate gyrus (FIG. 1c). Although adult SGZ RGLs have the capacity to give rise to all three neural lineages, under physiological conditions they produce neurons and astrocytes but not oligodendrocytes[21]. In young adult rodents, more than 30,000 neuroblasts exit the SVZ for the rostral migratory stream[22] and 9,000 new cells are generated in the dentate gyrus each day[23], demonstrating the robust neurogenic activity and large-scale plasticity that occur constitutively in these two regions.

Box 1 | **MicroRNAs and long non-coding RNAs in neurogenesis**

MicroRNAs (miRNAs) are a class of 20–25-nucleotide-long non-coding RNAs that regulate the stability and translation of their target mRNA through binding to the 3′ untranslated region (UTR) or the coding sequence of the given mRNA[154] (see the figure, part **a**). miRNAs have been found to regulate a variety of biological processes, including neurogenesis[154]. For instance, in embryonic neurogenesis, miR-19 promotes the proliferation of neural progenitor cells (NPCs) and the expansion of radial glial cells (RGCs) by targeting phosphatase and tensin homologue (*Pten*)[159]. Furthermore, miR-17-92 cluster inhibits T-box brain protein 2 (*Tbr2*) expression and prevents the transition of RGCs to neuronal intermediate progenitor cells (IPCs)[160] (FIG. 1a). By contrast, miR-184, let-7b, miR-137, miR-9 and miR-124 exert modulatory influences on adult neurogenesis by targeting various neuronally expressed genes[161–166]. Methyl-CpG-binding domain protein 1 (MBD1) promotes miR-184 expression, which in turn downregulates *Mbd1* mRNA levels to form a negative feedback loop. High levels of miR-184 promote the proliferation and inhibit the differentiation of NPCs[165]. In contrast to miR-184, let-7b promotes neural differentiation by targeting the stem cell regulator *Tlx* and *Ccnd1* (which encodes cyclin D1). Overexpression of let-7b enhances neuronal differentiation[164]. miR-137 is highly enriched in brains and promotes neural stem cell (NSC) differentiation by reducing the level of lysine-specific histone demethylase 1 (*Lsd1*) mRNA, which in turn downregulates miR-137 transcription[163]. miR-9 and *Tlx* form a similar feedback loop to promote premature neuronal differentiation[162]. miR-124 promotes NSC differentiation by inhibiting *Sox9*, as knockdown of miR-124 maintains the NSC state in the subventricular zone (SVZ)[166].

Long non-coding RNAs (lncRNAs) usually possess more than 200 nucleotides and function under different molecular mechanisms[167]. A pioneering study found that in the developing mouse forebrain the lncRNA *Evf2* recruits the transcription factors homeobox DLX proteins and methyl-CpG-binding protein 2 (MeCP2) to the intergenic regions of *Dlx5* and *Dlx6* to modulate their expression in both *trans*- and *cis*-acting manners (see the figure, part **b**) and *Evf2*-mutant mice have reduced numbers of GABAergic interneurons in the early postnatal hippocampus[168]. Large-scale lncRNA genome-wide profiling in the SVZ, olfactory bulb and dentate gyrus of mouse brains revealed a more tissue-specific pattern for lncRNAs than mRNAs[169]. Depletion of two lncRNAs identified in these regions, *Six3os* and *Dlx1as*, in SVZ NPCs leads to fewer newborn neurons[169].

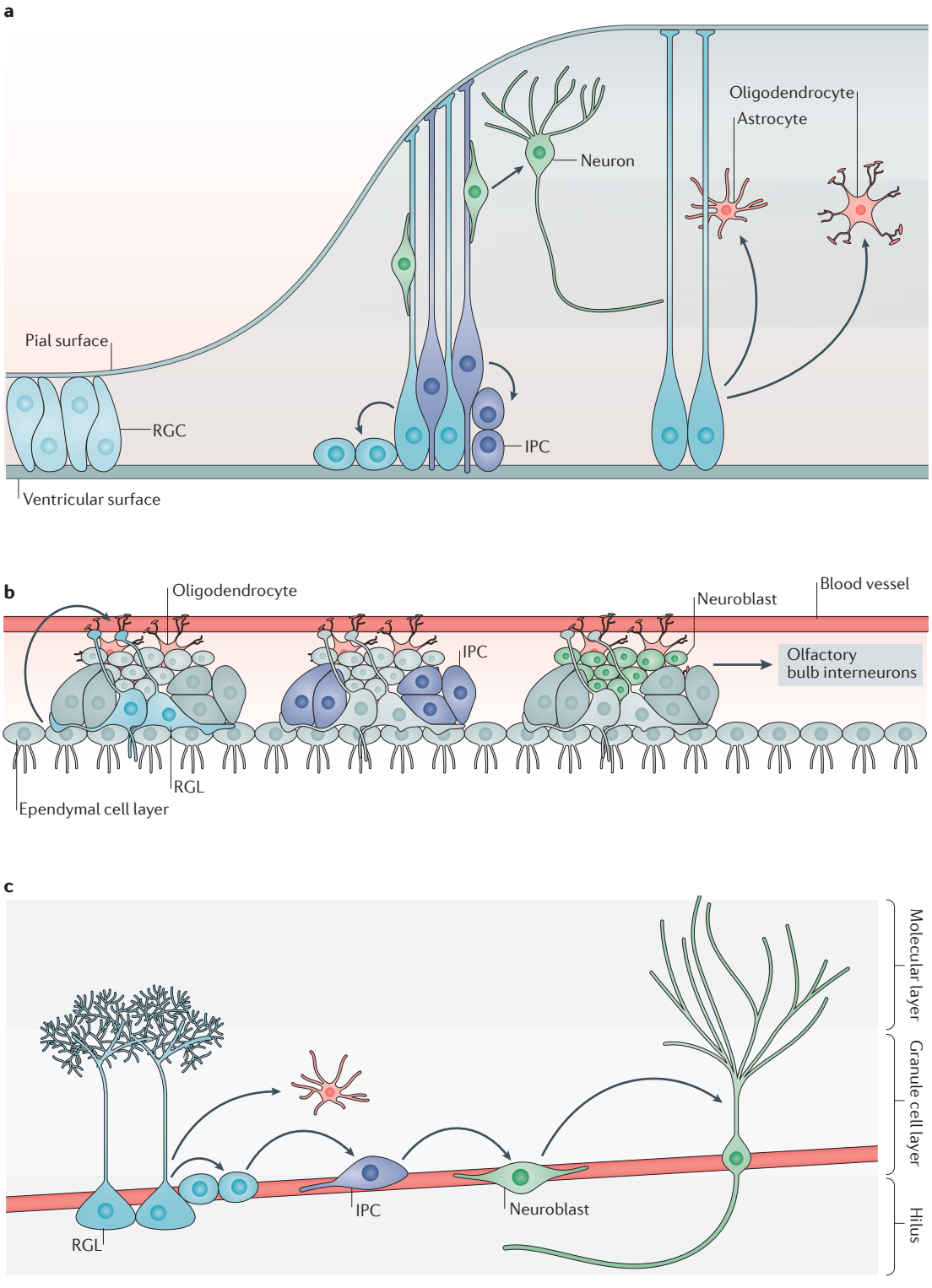



Embryonic neurogenesis establishes neural architecture and function on a global scale, whereas adult neurogenesis has a more restricted role: for example, in directly modulating the function of the hippocampus, a region that is essential for many forms of learning, memory and mood regulation[24]. To different extents, aberrant neurogenesis in both early development and adulthood appears to contribute to neurological and psychiatric disorders. Thus, it is crucial to understand the underlying molecular mechanisms. Most basic principles of neurogenesis are conserved between embryonic and adult stages, including the fundamental processes of stem cell differentiation[9]. However, the key feature of adult NPCs that distinguishes them from most embryonic NPCs is that they undergo long-term maintenance in a quiescent state within a neurogenic niche[18]. This property of adult NPCs is commonly found in stem cells from other adult somatic tissues and is a potential mechanism to regulate tissue homeostasis. Epigenetic modifications (FIG. 2a), which occur in response to both intrinsic signals and extracellular environmental cues, have important roles in maintaining NPCs and dictating their lineage commitments by gating the spatial and temporal expression of key regulators. For example, the influence of intrinsic factors is often mediated through epigenetic regulators, including writers, readers and erasers of DNA and histone modifications, as well as transcription factors[25]. In addition, the release and uptake of extracellular signalling molecules, such as growth factors, neurotrophins, cytokines and hormones, are under tight epigenetic control. Below, we focus on epigenetic regulatory control of neurogenesis at the molecular level.

## DNA methylation in neurogenesis

DNA methylation involves the chemical covalent modification of the 5-carbon position of cytosine: that is, the production of 5-methylcytosine (5mC) (FIG. 2b). Traditionally, studies of DNA methylation have focused on regions that contain a high frequency of CG dinucleotides, which are known as CpG islands[26]. In mammals, most CpG islands are hypomethylated, which ensures genomic stability, imprinted gene silencing and X-inactivation. Interestingly, recent studies have shown that most of the dynamic DNA methylation in neurons does not occur at CpG islands and instead takes place in regions with low CpG densities[27].

DNA methylation is catalysed by a family of DNA methyltransferases (DNMTs) that are responsible for preserving or generating 5mCs on the genome[28] (FIG. 2b). DNMT1 primarily functions to copy the existing methylation patterns during DNA replication for inheritance, whereas DNMT3A and DNMT3B work as *de novo* methyltransferases to generate new methylation patterns[28]. In the embryonic mouse CNS, *Dnmt1* is ubiquitously expressed in both proliferating NPCs and differentiated neurons[29]. *Dnmt3a* is expressed in SVZ NSCs starting from E10.5 and in postnatal neurons of almost all brain regions[30]. By contrast, *Dnmt3b* is robustly expressed in the SVZ between E10.5 and E13.5, but then its expression gradually diminishes and it becomes undetectable after E15.5 (REF. 30).




Radial glial cells
(RGCs). Bipolar cells derived from neuroepithelial cells during embryonic stages that primarily serve as neural progenitor cells during embryonic neurogenesis.

Imprinted gene silencing
A subset of genes that display a parental-specific expression pattern. Compared with normal genes, for which both paternal and maternal alleles are expressed, imprinted genes only express one parental allele. The silencing of one imprinted allele is often mediated by epigenetic mechanisms, such as DNA methylation.

X-inactivation
Females carry two copies of the X chromosome and therefore could potentially express toxic levels (a 'double dose') of X chromosome-linked genes. To prevent this scenario, cells of an early female embryo will randomly inactivate one of the two X chromosomes for gene dosage compensation, termed X-inactivation.

Figure 1 | **Embryonic and adult neurogenesis. a** | During embryonic neurogenesis in mice, neuroepithelial cells are activated around embryonic day 8 (E8) and develop into radial glial cells (RGCs) around E14. RGCs can either give rise to neurons directly or generate intermediate progenitor cells (IPCs), which in turn produce neurons. Later in development, RGCs also generate astrocytes and oligodendrocytes. **b** | Radial glia-like neural stem cells (RGLs) in the subventricular zone (SVZ) generate transient amplifying IPCs, which produce neuroblasts that migrate through the rostral migratory stream and become interneurons in the olfactory bulb. RGLs also produce oligodendrocytes. **c** | In the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, activation of quiescent RGLs gives rise to IPCs, which in turn produce neuroblasts that migrate along blood vessels and differentiate into dentate granule neurons. In addition, RGLs can give rise to astroglia in the adult dentate gyrus, and actively suppress an oligodendrocyte fate.

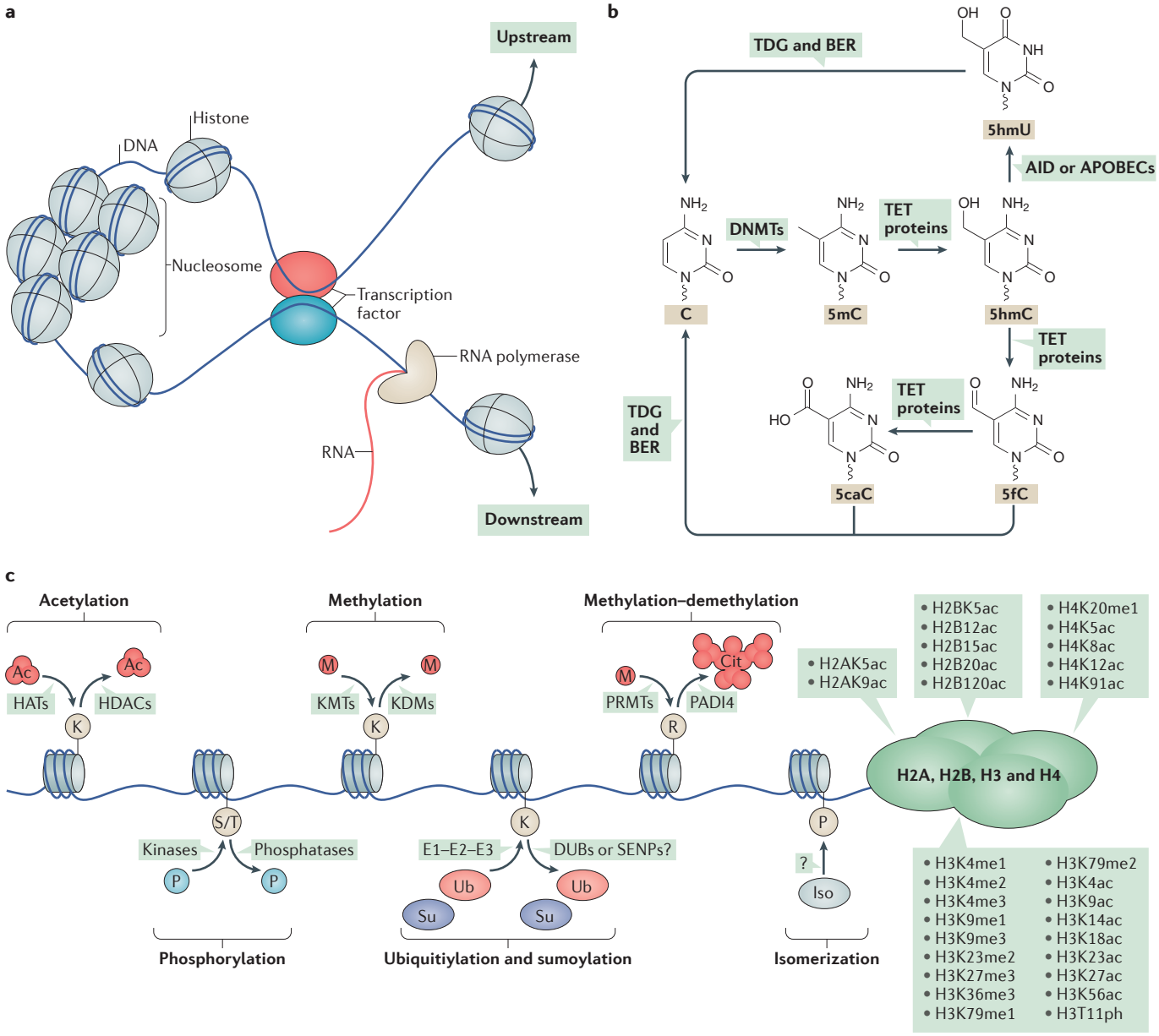



Figure 2 | **Major forms of epigenetic modifications. a** | Schematic illustration of chromatin organization in the nucleus. DNA is packaged into a highly ordered chromatin structure in eukaryotes by wrapping around an octamer of histone proteins, consisting of two copies of histone variants. **b** | DNA can be dynamically modified. Cytosines can be methylated by DNA methyltransferases (DNMTs) to 5-methylcytosine (5mC), which in turn can be oxidized to become 5-hydroxymethylcytosine (5hmC) by ten-eleven translocation (TET) proteins. 5hmC can be further oxidized by TET proteins to become 5-formylcytosine (5fC) and then 5-carboxylcytosine (5caC), or deaminated by activation-induced cytidine deaminase (AID) or apolipoprotein B mRNA-editing enzyme catalytic polypeptides (APOBECs) to become 5-hydroxymethyluracil (5hmU). 5fC, 5caC and 5hmU can be excised by thymine DNA glycosylase (TDG) to generate an abasic site, which can be converted back to a cytosine by the base excision repair (BER) pathway. **c** | Histone proteins can be modified in diverse ways. Various forms of histone modifications, including histone lysine and arginine methylation, lysine acetylation, ubiquitylation, sumoylation, serine and threonine phosphorylation, and proline isomerization, are indicated. Prevalent histone modifications that regulate gene expression are also listed. Cit, citrulline; DUBs, deubiquitylating enzymes; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; HATs, histone acetyltransferases; HDACs, histone deacetylases; KDMs, lysine demethylases; KMTs, lysine methyltransferases; PADI4, peptidyl arginine deiminase type 4; PRMTs, protein arginine methyltransferases; SENPs, sentrin-specific proteases.

A mutation in any of the three major *Dnmt* genes in mice leads to severe developmental abnormalities and embryonic, or early postnatal, lethality[31,32]. Deletion of *Dnmt1* specifically in embryonic NPCs results in hypomethylation and derepression of genes related to neuronal differentiation, including the astroglial marker gene glial fibrillary acidic protein (*Gfap*) and Janus kinase (JAK)–signal transducer and activator of transcription (STAT) astrogliogenic pathway genes, resulting in premature glial differentiation[33]. *Dnmt3a*-null mice

survive birth but have impaired postnatal neurogenesis compared with wild-type animals, produce tenfold fewer neurons and die in early postnatal stages[34]. Genome-wide analyses of DNMT3A-binding sites and DNMT3A-mediated site-specific DNA methylation in embryonic NPCs have revealed its direct epigenetic regulation of many neurogenic genes. In addition, through crosstalk with H3 lysine 27 trimethylation (H3K27me3), a repressive histone modification, and associated Polycomb group (PcG) protein modifiers, DNMT3A antagonizes H3K27me3-mediated gene repression[35]. Depletion of DNMT3B in the neuroepithelium promotes NPC differentiation instead of proliferation[36]. Together, these results indicate crucial and divergent roles of DNMTs and DNA methylation in different stages of neurogenesis. Further studies are needed to understand the genomic targets of different DNMTs and their context-dependent roles.

DNMTs can also methylate cytosines that are not adjacent to guanines in DNA *in vitro*[37], and an *in vivo* analysis revealed the presence of non-CpG (CpH, where 'H' can be an adenosine, a cytosine or a thymine nucleotide) methylation in mouse and human brains[38,39]. A recent study resolved the neuronal DNA methylome at single-base resolution from a relatively homogeneous population of mouse dentate granule neurons and showed that 75% of DNA methylation occurs at CpG sites, with the rest occurring at CpH sites[38]. Intriguingly, CpH methylation occurs *de novo* during neuronal maturation in both mice and humans[38,39]. Acute knockdown of DNMT3A in neurons leads to a loss of methylation at many CpH sites but not at CpG sites, suggesting that neuronal CpH methylation is more dynamic and actively maintained by DNMT3A[38]. Furthermore, CpH methylation seems to be a repressive epigenetic mark that uses methyl-CpG-binding protein 2 (MeCP2) as one of its readers[38,40,41]. As the mammalian CNS is highly heterogeneous and epigenetic modulations are cell type specific, a recent study examined purified specific populations of neuronal nuclei from adult mouse brains. It confirmed that CpH methylation is a common feature in different neuronal subtypes and found that transcriptional repression is more strongly correlated with CpH methylation in promoters and intragenic regions than with CpG methylation[42]. Thus, CpH methylation, in contrast to traditional CpG methylation that remains stable and repressive, could function as a flexible and dynamic form of epigenetic regulation, particularly in mammalian brains. The precise differences between CpG and CpH methylation in terms of their roles in transcriptional regulation, however, remain to be determined.

Once DNA methylation marks are established, a set of methyl-CpG-binding proteins function as readers to interpret the 5mC signal and mediate its function. Methyl-CpG-binding domain protein 1 (MBD1) preferentially binds to hypermethylated CpG islands in gene promoter regions, and its depletion impairs adult hippocampal neurogenesis and genomic stability *in vitro*[43]. MBD1 occupies and protects the methylation of the promoter for basic fibroblast growth factor 2 (*Fgf2*), which encodes a growth factor essential for neural development. Loss of MBD1 leads to hypomethylation and derepression of *Fgf2* in NPCs, resulting in the failure of these cells to differentiate[44]. MeCP2 was originally identified as a specific methyl-CpG-binding protein[45], but was later found to bind to other modified cytosines[38,40,46]. Similar to *Dnmt3a*-null mice, *Mecp2*-knockout mice exhibit much delayed and impaired neuronal maturation compared with wild-type mice, with higher expression levels of several genes related to synaptic development in the dentate gyrus[47]. One well-characterized MeCP2 target is brain-derived neurotrophic factor (*Bdnf*)[48,49], which encodes a protein that promotes the growth and differentiation of newborn neurons.

In addition to methyl-CpG-binding proteins as DNA methylation readers, many transcription factors exhibit specific binding to methylated and unmethylated DNA motifs of distinct sequences[50]. Therefore, in contrast to the prevailing view that 5mC nucleotides prevent transcription-factor binding, DNA methylation increases the diversity of binding sites for transcription factors. Many of these transcription factors, such as recombining binding protein suppressor of hairless (RBPJ), Fez family zinc finger protein 2 (FEZF2) and myocyte-specific enhancer factor 2A (MEF2A), are well known to regulate neurogenesis[51]. It will be interesting to examine the binding specificities of these transcription factors and their effects on gene expression during neurogenesis.

## DNA demethylation in neurogenesis

DNA methylation can be 'passively diluted' via cell division, but mechanisms of active removal of DNA methylation have only recently been discovered. Ten-eleven translocation 1 (TET1) was shown to catalyse the conversion of 5mC to 5-hydroxymethylcytosine (5hmC)[52] (FIG. 2b). Subsequent studies revealed that three TET family proteins could further oxidize 5hmC to 5-formylcytosine (5fC) and then to 5-carboxylcytosine (5caC)[53–55]. In addition, 5hmC can be converted to 5-hydroxymethyluracil (5hmU) by the deaminases activation-induced cytidine deaminase (AID; also known as AICDA) and apolipoprotein B mRNA-editing enzyme catalytic polypeptides (APOBECs)[56]. All of these derivatives (5fC, 5caC and 5hmU) can be successively excised by thymine DNA glycosylase and replaced by an unmodified cytosine through the base-excision repair pathway to complete the active DNA demethylation process[3] (FIG. 2b).

***DNA demethylation derivatives in neurogenesis.*** The role of active DNA demethylation in neurogenesis was initially suggested by the finding that growth arrest and DNA-damage-inducible protein 45β (GADD45β) promotes adult hippocampal neurogenesis[57]. GADD45 family members have been implicated in active DNA demethylation in various systems[58,59]. GADD45B enhances promoter DNA demethylation and the expression of several genes, including *Bdnf* and *Fgf1*, in dentate granule neurons, which in turn promotes NPC proliferation and new neuron development in a neuronal activity-dependent manner in the adult mouse hippocampus[57]. Next-generation high-throughput sequencing technology

TET family proteins
Ten-eleven translocation (TET) proteins serve as methylcytosine dioxygenases to convert 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine in an iron-dependent manner.

DNA demethylation
An active biochemical process that removes a methyl group from cytosine; this process is catalysed by methylcytosine dioxygenases, such as ten-eleven translocation (TET) proteins.

has spurred the rapid development of genome-wide mapping of cytosine modification derivatives in many different cell types and tissues[60]. Genome-wide profiling revealed that 5hmC accumulates during embryonic neurogenesis, as NPCs give rise to mature neurons, and its overall level continues to rise during ageing[61,62]. By contrast, the differentiation of embryonic stem (ES) cells into embryoid bodies causes a marked reduction in 5hmC. Interestingly, the acquisition of 5hmC in several developmentally activated genes does not coincide with a concomitant demethylation of 5mC to unmethylated cytosines, suggesting that 5hmC could itself serve as an epigenetic signal[61,62]. Although the exact relationship between 5hmC distribution and gene expression is still under debate, cell type-specific active gene transcription coincides with enriched 5hmC and depleted 5mC on gene bodies[46]. In addition, 5hmC interacts with other epigenetic mechanisms, such as histone modifications, to regulate neurogenesis[61]; however, the exact mechanism remains to be elucidated.

5fC and 5caC are primarily considered to be DNA demethylation intermediates, owing to their extremely low abundance in the genome. Genome-wide mapping of 5fC and 5caC indicates that 5fC is preferentially found in distal regulatory regions, such as poised enhancers[63]. Considering that 5hmC has also been suggested to mark regulatory regions[64], active DNA demethylation in these regions may facilitate specific transcriptional regulation. A recent report systematically quantified 5fC in various mouse tissues and revealed its relatively high abundance in brains over other tissue types, raising the possibility that 5fC could also serve as a stable epigenetic modification[65].

One approach to address the independent functions of these DNA demethylation derivatives is to identify their potential reader proteins. MeCP2 was recently reported to also bind to 5hmC *in vitro*[40,46]. A large-scale quantitative proteomics analysis identified numerous binding proteins for different cytosine variants in ES cells, embryonic NPCs and adult mouse brain tissue[66]. Further analyses revealed partially overlapping readers of cytosine derivatives that can selectively bind to distinct derivatives within different cellular contexts. For example, UHRF2, an E3 ubiquitin-protein ligase, specifically binds to 5hmC in NSCs[67]. In the mouse brain, the homeobox protein DLX1 exclusively interacts with 5mC, whereas WD repeat-containing protein 76 (WDR76) and thymocyte nuclear protein 1 (THYN1; also known as THY28) are 5hmC-specific readers. Further studies are needed to address the functional impact of these interactions in regulating neurogenesis.

***TET proteins in neurogenesis.*** TET proteins initiate active DNA demethylation via oxidation of 5mC into 5hmC (REF. 3). Different isoforms of TET proteins appear to have different preferences for genomic sites to demethylate. For example, TET1 depletion diminishes 5hmC levels at transcription start sites, whereas TET2 depletion is primarily associated with gene-body 5hmC depletion in mouse ES cells[68]. TET proteins have a dual role in both activation and repression of their target genes, depending on the cofactors that they bind to or on their interactions with other epigenetic modifiers. Among TET-binding partners, *O*-GlcNAc transferase subunit p110 (OGT), homeobox protein Nanog and poly(ADP-ribose) polymerase 1 (PARP1) may be involved in TET-mediated gene activation, whereas paired amphipathic helix protein SIN3A serves as a co-repressor for TET-mediated gene silencing[3].

Recent studies have explored the functions of TET proteins and their cofactors in neurogenesis. *Tet1*-knockout mice exhibit a decrease in the number of NPCs in the adult SGZ, and NPCs isolated from these mice show reduced proliferation when grown as neurospheres[69]. Several genes, including those involved in adult NPC proliferation (for example, galanin (*Gal*), chondroitin sulfate proteoglycan 4 (*Cspg4*; also known as *Ng2*) and neuroglobin (*Ngb*)), exhibit hypermethylation and reduced expression in NPCs following *Tet1* deletion *in vitro*[69]. Depletion of tet3 in *Xenopus laevis* embryos, mediated by morpholino antisense oligonucleotides, represses many key developmental genes, such as *Pax6*, neurogenin 2 (*Ngn2*) and *Sox9* (REF. 70). Together, these findings indicate that TET proteins have independent but interactive roles in neurogenesis.

## Histone modifications

DNA is packaged into a highly ordered chromatin structure in eukaryotes by wrapping around an octamer of histone proteins, which consists of two copies of histone variants, including H2A, H2B, H3 and H4 (REF. 60) (FIG. 2a). Chemical covalent modifications of the amino acids on the amino-terminal 'histone tails' define the transcriptional environment by serving as docking stations to attract various epigenetic modifiers and transcription factors for transcriptional modulation. In addition, crosstalk between histone and DNA modifications has been suggested to coordinate the patterning and maintenance of the transcriptome landscape[60] (FIG. 2c). It is well established that histone methylation and acetylation on lysine residues have fundamental roles in neurogenesis[9]. As histone modifications in neurogenesis have been extensively reviewed elsewhere[71–74], we only focus on a few examples of histone methylation and acetylation in neurogenesis.

***Histone methylation and demethylation in neurogenesis.*** Histone methylation can occur on basic residues such as lysine and arginine, and these amino acids are subject to multiple methylations on their side chains[75]. Many histone modifications, such as H3K4me3 and H3K27me3, have been found to be associated with active or repressive transcription, respectively (FIG. 2c). Dynamic methylation of lysine residues can be mediated by a range of lysine methyltransferases (KMTs) as writers and lysine demethylases as erasers[75]. Many proteins possess KMT properties, including the well-known PcG repressive complex (PRC) and Trithorax active complex (TRXG)[75,76]. Enhancer of zeste homologue 2 (EZH2) in PRC2 is responsible for generating the repressive mark H3K27me3, which can be further bound by the PRC1 complex to maintain transcription repression[76]. By contrast, mixed-lineage

Poised enhancers
Enhancers refer to the genomic regions that are characterized by uniquely bound transcription factors such as P300 and signature histone modifications such as histone H3 lysine 4 methylation (H3K4me1) that could potentially modulate transcription activation. Poised enhancers bear enhancer characteristics, but their functions are hampered by repressive chromatin marks such that they require additional cues to unleash their functions.

Transcriptome landscape
Global signature transcriptional patterns of different cell types. Maintaining cell type-specific gene expression is crucial for cell identity.

leukaemia 1 (MLL1; also known as KMT2A) in TRXG counteracts PRCs and generates H3K4me3 to reverse transcription states[77].

Both PcG proteins and TRXG have been implicated in the regulation of neurogenesis. During embryonic neurogenesis, PcG proteins control the neurogenic to astrogenic transition of NPCs by modulating the expression of *Ngn1*, a neurogenic gene[78]. Deletion of *Ezh2* in embryonic cortical NPCs results in a global loss of H3K27me3, derepression of a large set of neuronal genes and impaired neuronal differentiation[79]. Deletion of *Bmi1*, which encodes an oncogenic protein that forms part of PRC1, derepresses the cell cycle inhibitor $p16^{INK4A}$ and reduces the size of the NPC population both *in vitro* and *in vivo*, whereas its overexpression leads to the promotion of the SVZ NPC state[80]. These observations suggest that BMI1 maintains neurogenesis homeostasis by balancing the cell lineage-specific transcriptomes. As an active histone modulator, MLL1 in TRXG is highly enriched in the SVZ and its depletion inhibits neuronal differentiation. Mechanistically, TRXG proteins preserve the expression of DLX2 by maintaining H3K4me3 on its gene promoter[81]. Intriguingly, depletion of MLL1 causes the enrichment of both H3K27me3 and H3K4me3 on the *Dlx2* promoter[81]. Bivalent marks have been found to coexist on many genes in ES cells that are poised to be expressed upon differentiation[82], suggesting that they have collaborative roles in the precise control of spatial and temporal gene expression. It has also been suggested that some neuron-specific genes acquire bivalent marks when ES cells become NPCs, so that these genes remain repressed but primed for expression upon neuronal differentiation[83].

A set of histone demethylases have been identified that remove specific histone methylations on specific loci. Lysine-specific histone demethylase 1 (LSD1; also known as KDM1A), the first histone lysine demethylase to be identified, selectively demethylates H3K4me2 and H3K4me1, and knockdown of LSD1 severely impairs NPC proliferation in the adult dentate gyrus[84]. An isoform of LSD1 (LSD1+8a) mediates H3K9me2, instead of H3K4me2, demethylation to regulate neuronal differentiation[85]. Jumonji domain-containing protein 3 (JMJD3; also known as KDM6B), which belongs to another class of H3K27me3 demethylases, has also been implicated in neurogenesis[86]. Enhanced expression of JMJD3 promotes demethylation of several neuronal genes, including neuronal migration protein doublecortin (*Dcx*), NK2 homeobox 2 (*Nkx2.2*) and *Dlx5*, which induces neuronal differentiation. Taken together, these results demonstrate that proper histone methylation and demethylation dynamics need to be tightly regulated to ensure the precise control of gene expression during neurogenesis in the mammalian CNS.

***Histone acetylation and deacetylation in neurogenesis.*** Histone acetylation is catalysed by histone acetyltransferases (FIG. 2c). Similar to histone methylation, histone acetylation is a reversible process — which is triggered by histone deacetylases (HDACs) — and is involved in neurogenesis[87]. One well-characterized histone acetyltransferase is KAT6B (also known as protein querkopf in mice), which is highly enriched at the protein level in the adult SVZ. A lack of KAT6B leads to a reduction in the number of migrating neuroblasts in the rostral migratory stream and a marked reduction in the number of olfactory bulb interneurons[88]. Similarly, mutations in *Kat6b* impair embryonic cerebral cortex development[74].

A collection of more than 18 HDACs modulates histone deacetylation in the mammalian genome in a tissue-specific manner. For example, HDAC2 is upregulated during the differentiation of NSCs into neurons, whereas HDAC1 is found primarily in glial cells in the adult brain[89]. TLX (also known as NR2E1), a transcription factor that has a crucial role in NSC proliferation and self-renewal, recruits HDACs to target loci, such as *P21* and phosphatase and tensin homologue (*Pten*), which positively influence neuronal growth[90]. Ankyrin repeat domain-containing protein 11 (ANKRD11) is a chromatin regulator implicated in autism and neural development. By interacting with HDAC3, ANKRD11 regulates neurogenesis-related genes, and its knockdown results in a decrease in precursor proliferation[91]. Despite impressive progress, a comprehensive picture of the involvement of HDACs in neurogenesis requires further investigation.

Development of many pharmacological HDAC inhibitors allows for manipulating histone acetylation-mediated biological processes. Given that histone acetylation may have broad epigenetic roles in gene expression, HDAC inhibitors are likely to be pleiotropic and, as such, may influence transcriptomic changes related to neurogenesis directly or indirectly. For example, trichostatin A, a well-known HDAC inhibitor, is reported to impair neurogenesis in the ganglionic eminences but triggers a modest increase in neurogenesis in the cortex[92]. Mechanistically, trichostatin A simultaneously promotes bone morphogenetic protein 2 (BMP2) expression while inhibiting SMAD7 expression to shift the neurogenic balance and control lineage specificity. Valproic acid, another HDAC inhibitor, promotes the differentiation of adult hippocampal NPCs but inhibits astrocyte and oligodendrocyte differentiation, at least in part by inducing the expression of neurogenic differentiation factor 1 (NeuroD)[93]. Notably, lysine acetylation occurs in various proteins[94], in addition to histones. Therefore, these HDAC inhibitors may exhibit a broad influence over neurogenesis through direct or indirect epigenetic manipulations.

## Epigenetic dysregulation in brain disorders

Given the crucial roles of adult neurogenesis in many aspects of brain function, such as cognitive abilities and mood regulation, it is not surprising that its dysregulation may contribute to various brain disorders[24]. Cumulative evidence now suggests that epigenetic dysregulation also plays an important part in many of these same disorders. Here, we focus on how epigenetic mechanisms may contribute to aberrant expression of risk-associated genes, and on the impact of such aberrant expression on neurogenesis and disease pathogenesis.

***Adult neurogenesis in neurodegenerative disorders.*** Several animal models of degenerative neurological disorders, including Parkinson disease (PD), Alzheimer disease (AD) and Huntington disease (HD)[95], exhibit significant impairments in adult neurogenesis. Dopamine depletion, a hallmark of PD, reduces SGZ NPC proliferation in adult rodents[96], and post-mortem analyses of brains from individuals with PD revealed decreases in proliferating cells in the dentate gyrus[96]. Transgenic mice carrying mutations in or overexpressing PD-related genes, such as *SNCA* (which encodes α-synuclein) and leucine-rich repeat kinase 2 (*LRRK2*), recapitulate many of the hallmark phenotypes of this disorder[95,97–99].

α-Synuclein is specifically enriched in presynaptic terminals and coordinates with cysteine string protein-α (also known as DNAJC5) to stimulate neurogenesis, to maintain synaptic integrity and to prevent neurodegeneration[100]. *SNCA* expression needs to be precisely regulated, as high levels of α-synuclein, which are often found in the brains of patients with PD, can lead to increased cell death and impaired dendritic development of newborn neurons in the adult mouse hippocampus[101]. *SNCA* transcription is subject to epigenetic modulation, as CpG islands in intron 1 of *SNCA* become hypomethylated in PD, resulting in *SNCA* overexpression[102,103]. Interestingly, DNMT1 is systematically relocated from the nucleus into the cytoplasm in both human post-mortem PD brains and brains of *SNCA* transgenic mouse models. Nuclear DNMT1 depletion is responsible for hypomethylation of many PD-related genes, including *SNCA*. DNMT1 relocation results from sequestration by α-synuclein, and DNMT1 overexpression in transgenic mouse brains partially restores nuclear DNMT1 levels[104].

*LRRK2* encodes a multidomain protein with GTPase and kinase activities; overexpression of the human *LRRK2* G2019S mutation, which causes PD symptoms, severely impairs the survival of newborn neurons in the mouse dentate gyrus and olfactory bulb, and reduces dendritic arborization and spine formation[105]. Recent evidence also indicates the importance of appropriate *LRRK2* transcriptional control for a range of brain functions. Global overexpression of wild-type *LRRK2* leads to altered short-term synaptic plasticity, behavioural hypoactivity and impaired recognition memory[106]. *LRRK2* is post-transcriptionally regulated by miR-205 (REF. 107). Through direct targeting of the 3′ untranslated region of *LRRK2*, miR-205 inhibits the translation and controls the levels of LRRK2. Expression of miR-205 is markedly downregulated in individuals with sporadic PD and is associated with an increase in LRRK2 levels. The introduction of precursor miR-205 into hippocampal neurons carrying a *LRRK2* R1441G mutation, which is related to PD, rescues the neurite outgrowth defects[107]. These findings highlight that the epigenetic regulation of PD risk genes may contribute to PD pathogenesis through its effects on adult neurogenesis.

AD features extensive neurodegeneration in the forebrain and cortex and is associated with two hallmark pathologies: neurofibrillary tangles, which are caused by tau protein phosphorylation, and amyloid plaques[108]. Genetic studies have identified various risk factors associated with early- or late-onset AD. For instance, mutations in amyloid precursor protein (*APP*) and two presenilin genes (*PSEN1* and *PSEN2*) are highly associated with early-onset AD, whereas polymorphisms in apolipoprotein E (*APOE*) are linked to late-onset AD[109,110]. Studies from several transgenic AD mouse models bearing either mutations in or overexpression of these high-risk genes have demonstrated altered neurogenesis processes[95]. The first transgenic AD mouse was developed 20 years ago by expressing human *APP* and showed a phenotype resembling aspects of AD[111]. Another AD transgenic mouse model bearing overexpression of the Swedish double mutant form of APP695 also showed AD phenotypes, such as amyloid plaques[112]. The most common pan AD mouse model, the triple transgenic mice (3xTg-AD), was generated by expressing mutant *APP*, *PSEN1* and *MAPT* (which encodes tau). Cumulative evidence now indicates that epigenetic dysregulation of *APP*, *PSEN1*, *PSEN2, APOE* and/or *MAPT* could potentially contribute to AD pathogenesis[113]. For example, a global decrease in DNA methylation and hydroxymethylation levels in the hippocampus of patients with AD has been reported[114]. A recent genome-wide DNA methylation analysis of AD brains revealed altered DNA methylation states of 71 discrete CpG dinucleotides, which were accompanied by dysregulated expression of associated genes[115]. It has been known that PSEN1 is required for neurodevelopment and differentiation, as lack of *Psen1* causes premature NPC differentiation[116]. Loss of *Psen1* also induces learning and memory deficits in mice that appear to owe to impaired adult hippocampal neurogenesis[117]. Promoter DNA methylation, in coordination with H3K9 acetylation, controls the expression of *Psen1* in the cerebral cortex during development[118]. In *Apoe*-knockout mice, hyperactive BMP signalling promotes glial differentiation during neurogenesis[119]. Recent studies propose that hypermethylated CpG islands in the 3′ end of *APOE* possess dual regulatory roles as either enhancers or silencers to regulate the transcription of multiple genes, including *APOE*, *TOMM40* and *NEDD9* (REF. 120).

HD is a progressive brain disorder that results from a greater than 41 CAG trinucleotide repeat expansion in huntingtin (*HTT*)[121]. The CAG repeats in the coding sequence generate a mutant HTT protein that contains a long polyglutamine tract, causing intranuclear and perinuclear aggregates in neuronal cells[122]. HD mouse models expressing mutated *HTT* exhibit reduced NPC proliferation in the adult dentate gyrus, resulting in fewer newborn neurons[123,124]. Although no obvious defect in NPC proliferation has been observed in the adult SVZ, there is a reduction in adult-born neurons in the olfactory bulb, where HTT aggregates[125]. Convergent evidence suggests an important role for epigenetic modulation in HTT-mediated effects on neurogenesis[126]. Epigenetic mechanisms could directly affect the expression or the expansion length of *HTT*, or mutant HTT could alter epigenetic states by interacting with numerous epigenetic modulators. For example, methylation of the repeat sequence has been shown to effectively prevent the generation of long expansion repeats *in vitro*, and treating cells with a DNMT inhibitor triggers global

demethylation and promotes the generation of longer repeat expansion during replication[127]. Comparing the direct impact on the repeat expansion in HTT, an analysis of wild-type and mutated HTT interactomes reveals their differential participation in biological networks, many of which are related to epigenetic modulations, suggesting that mutated HTT could ectopically influence cellular epigenetic states[128,129]. For example, mutant HTT significantly interacts with the RNA helicase DHX9 (also known as RHA) in the mouse cortex, which could potentially change the global transcriptome[129]. Genome-wide DNA methylation analysis suggests that the expression of mutant HTT triggers large-scale changes in DNA methylation and in the transcriptome, and has an effect on many genes that are crucial for neurogenesis, such as downregulation of *Pax6* and *Nes* (which encodes nestin) in mice[130]. Furthermore, global 5hmC profiling in the striatum and the cortex of transgenic HD mice reveals a genome-wide loss of 5hmC, which is generally associated with decreased transcription. A 5hmC gene pathway analysis in HD mice revealed that many canonical biological pathways involved in neurogenesis might be affected in this disease possibly owing to the 5hmC alteration[72].

***Adult neurogenesis in psychiatric disorders.*** Neurodegenerative diseases such as HD, as well as adult neurogenesis itself, have been associated with psychiatric disorders[24,131–133]. For example, adult hippocampal neurogenesis has been implicated in major depressive disorders, and enhanced neurogenesis often parallels the success of various antidepressant treatments[134]. Similarly, antidepressant treatments increase adult hippocampal neurogenesis in both rodents and humans[135,136]. Several potential factors involved in the pathophysiology of major depression have also been linked to adult neurogenesis, such as BDNF[137]. Knockdown of *Bdnf* in the mouse dentate gyrus reduces hippocampal neurogenesis and affects depressive-like behaviours[137]. *Bdnf* expression is regulated by several epigenetic factors, including MeCP2, which is one of its best-characterized transcription modulators. However, the precise mechanisms through which MeCP2 regulates *Bdnf* expression are not well understood, and there are conflicting data from different laboratories using different models. Earlier studies using primary neuronal culture *in vitro* suggested a repressive role of MeCP2 in *Bdnf* expression[48,49]. Mechanistically, MeCP2 specifically recognizes methylated DNA at promoter IV of *Bdnf* and further recruits the transcription repressors SIN3A and HDAC1. Thus, DNA demethylation causes MeCP2 unbinding and therefore prevents MeCP2 from inhibiting *Bdnf* expression[48,49]. However, this model has been challenged by the finding that BDNF levels are lower in the brains of *Mecp2*-knockout mice than in those of wild-type mice[138] and by the report that MeCP2 overexpression, by blocking its inhibitor miR-132, activates *Bdnf* transcription in cortical neurons *in vitro*[139]. Furthermore, a recent study in cultured hippocampal neurons indicates that both MeCP2 knockdown and overexpression increase BDNF levels[140]. Considering that MeCP2 possesses dual roles in recognizing both 5mC and 5hmC, it is likely that it also has diametric influences on target genes such as *Bdnf*, depending on the cellular context and MeCP2-binding partners[141].

In addition to ectopic MeCP2 localization, mutations in X-linked *MECP2* cause Rett syndrome, a severe progressive neurodevelopmental disorder[142]. A number of mouse models with *Mecp2* mutations or conditional *Mecp2* knockouts recapitulate Rett syndrome phenotypes, and disruption of epigenetic regulatory processes has been thought to be the primary trigger for the onset of Rett syndrome[142]. Post-translational modifications of MeCP2, such as phosphorylations on specific amino acids, alter MeCP2 function and correlate with Rett syndrome onset. For example, brain-specific S421 phosphorylation of MeCP2 can be triggered by neuronal activity and the subsequent calcium influx, and controls the ability of MeCP2 to regulate dendritic patterning and morphology through transcriptional control of key genes, such as *Bdnf*[143]. A recent report showed that MeCP2 S421 phosphorylation modulates adult neurogenesis by controlling the balance between proliferation and neural differentiation through the Notch signalling pathway in NPCs isolated from the adult mouse hippocampus[144]. Given the diverse roles of MeCP2 in binding to 5mC, 5hmC and methyl-CpH, further studies are needed to address how specific interactions of MeCP2 with various epigenetic modifications lead to pathogenesis and aberrant neurogenesis in Rett syndrome.

Schizophrenia is a chronic, severe and disabling brain disorder that is usually accompanied by positive symptoms, such as hallucinations and delusions, as well as negative symptoms, including loss of pleasure and social withdrawal[145]. One study has reported decreased adult neurogenesis in post-mortem brains from people with schizophrenia, as indicated by fewer NPCs in the adult dentate gyrus[146]. The role of epigenetic regulation in schizophrenia has begun to be appreciated[147]. For example, an analysis of post-mortem brains from individuals with schizophrenia revealed alterations in DNA and histone modifications in crucial neuronal genes, such as reelin (*RELN*), glutamate decarboxylase 1 (*GAD1*) and *BDNF*[148–153].

Taken together, these studies clearly emphasize the crucial role of epigenetic regulation in neurogenesis and highlight that dysregulation of some of the same epigenetic processes are implicated in various neurological and psychiatric disorders.

## Concluding remarks

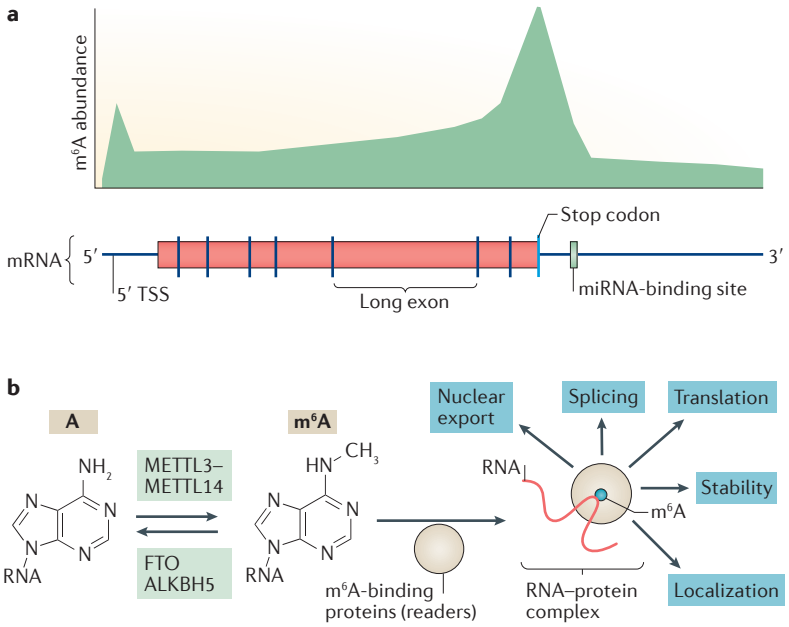
Many epigenetic mechanisms appear to be conserved across different cell types, including those in the nervous system. Emerging evidence, however, reveals that neurogenesis is associated with unique epigenetic features, such as the accumulation of CpH methylation during neuronal maturation and dynamic DNA modifications in neurogenesis and neuroplasticity. Although this Review focuses on modifications of DNA and histones, a growing body of work has demonstrated crucial roles for non-coding regulatory RNAs, including miRNAs and lncRNAs, in regulating embryonic and adult neurogenesis[154–156] (BOX 1). Furthermore, more than 100 post-transcriptionally

Interactomes
Whole sets of molecules that physically interact with given molecules. In this article, interactome specifically refers to protein–protein interactions.

Box 2 | **m$^6$A RNA methylation**

A relatively abundant modification of mRNA and long non-coding RNA (lncRNA) is *N*$^6$-methyladenosine (m$^6$A), which occurs at one in three adenosine residues in mammalian mRNA[4,170]. It is also reversible and dynamically regulated[4,170]. Three salient features of the m$^6$A methylome are conserved in mammals. First, m$^6$A sites are mainly confined to the consensus motif Pu[G>A]– m$^6$A–C[U>A>C][4]. Second, m$^6$A marks are not equally distributed across the transcriptome; rather, they are preferentially enriched in a subset of consensus sequences near stop codons, in 3′ untranslated regions (UTRs) and in long internal exons[171,172] (see the figure, part **a**). Third, m$^6$A-modified genes are well conserved between human and mouse embryonic stem (ES) cells and somatic cells[171,172]. In addition, different species or cells at different developmental stages can show distinct m$^6$A patterns[4].

Recent efforts have led to the identification of m$^6$A writers, erasers and readers[4] (see the figure, part **b**). In mammals, m$^6$A is installed by a three-protein core complex comprising two catalytic subunits, methyltransferase-like protein 3 (METTL3) and METTL14, and an accessory factor, Wilms tumour 1-associating protein (WTAP)[173–177]. m$^6$A on mRNA can be reversed by two RNA demethylases: fat mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alkB homologue 5 (ALKBH5)[178,179]. Several molecular mechanisms of m$^6$A-mediated gene regulation have been identified, including mRNA decay, microRNA (miRNA) production and translational control[158]. For example, an m$^6$A-specific reader, YTH domain-containing family protein 2 (YTHDF2), regulates the translocation of bound mRNA from translation pools to P-bodies for RNA decay[180]. Furthermore, methylation of mRNA could antagonize Hu-antigen R (HuR; also known as ELAVL1), an RNA-binding protein that recognizes AU-rich elements in the 3′ UTR of mRNA and facilitates miRNA-mediated gene silencing. Depletion of m$^6$A allows the association of HuR and stabilizes these transcripts in mouse ES cells[180]. Moreover, m$^6$A modification has been shown to change the molecular structure of RNA and alter its association with m$^6$A-binding proteins, such as YTHDF2, heterogeneous nuclear ribonucleoprotein C (HNRNPC)[181] and HNRNPA2B1 (REF. 182). Altered m$^6$A levels, which are due to loss of METTL3, affect HNRNPA2B1 association on primary miRNA transcripts and thus thousands of their downstream targets[182]. m$^6$A modification has also been shown to participate in translation regulation. The latest report shows that the adenosine methylation on the 5′ UTR of critical genes in response to heat shock can be protected by YTHDF2 and promotes cap-independent translation initiation[183]. Taken together, these findings strongly support an epigenetic role of RNA m$^6$A modification in regulating gene expression (see the figure, part **b**). Future studies are needed to address the specific role of dynamic m$^6$A modifications in regulating neurogenesis. TSS, transcription start site. Part **a** is from REF. 4, Nature Publishing Group.




modified ribonucleosides have been identified in various types of RNA[157]. Many RNA modifications have fundamental roles in regulating aspects of RNA metabolism, including splicing, transport, translation and decay[157,158] (BOX 2). These dynamic RNA modifications represent another level of gene regulation, termed 'epitranscriptomics'. Addressing the role of epitranscriptomics in neurogenesis will be an exciting new area to explore.

The rapid development of novel techniques, such as new next-generation high-throughput sequencing technologies, genomic editing and human brain organoid cultures, has brought us to an era of unprecedented opportunities to decipher brain development and functions. It is now feasible to investigate in detail the dynamic epigenetic and global transcriptome changes at single-cell resolution, including single-cell RNA sequencing (RNA-seq), assay for transposase-accessible chromatin using sequencing (ATAC-seq), Hi-C and chromatin immunoprecipitation followed by sequencing (ChIP–seq). Novel labelling techniques have further enabled us to identify and purify homogeneous populations of neuronal cells, minimizing confounds in findings that arise from the analysis of multiple cell types at various developmental stages. Future efforts should include generating a comprehensive map of dynamic epigenetic processes from NPCs to mature neurons at the single-cell level to understand the regulatory sequences that underlie cell fate decisions, neuronal development and cell type-specific functions, and how these processes may be dysregulated in neurological and psychiatric disorders.

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The authors declare no competing interests.