Epigenetic modifications are chemical changes to DNA and histones that modulate gene expression without altering the DNA sequence. A well-known epigenetic mark in mammals is the addition of a methyl group to cytosine, one of the four bases of DNA, which produces 5-methylcytosine (5mC). 5mC is found almost exclusively as a symmetrical mark in CG dinucleotides, and enzymes of the DNA (cytosine-5)-methyltransferase (DNMT) family catalyze its formation. In mammals, 5mC abounds throughout the genome and is crucial to complete embryonic development. Over the last few decades, several functions have been assigned to this epigenetic mark, such as the long-lasting silencing of genes and of parasitic mobile elements. As development proceeds, many loci undergo cell-type-specific changes in DNA methylation that ultimately contribute to the establishment and stabilization of a chosen gene expression program.

Another layer of complexity comes from the discovery that 5mC can be further oxidized by ten-eleven translocation (TET) proteins into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). These novel modified forms of cytosine are primarily viewed as intermediates of DNA demethylation reactions.

Intriguingly, DNA methylation behaves uniquely in the brain compared with other tissues. Recent studies revealed that genomic DNA from the adult brain contains high levels of 5mC in a non-CG context [1]. In addition, the levels of 5hmC in the brain dramatically exceed those observed in other tissues [2]. Genetic studies further indicated that changes in DNA methylation are important for brain development and learning. The loss of Dnmt1, Dnmt3A or Tet1 in the adult brain leads to cognitive deficits in mice [3,4]. In humans, mutations in DNMT1 are associated with a form of neurodegenerative disease [5], and mutations in methyl-CpG-binding protein 2 (MECP2), a protein that binds methylated DNA, are observed in several neurodevelopmental disorders [6]. Despite these indications that DNA epigenetic marks are crucial for cognitive functions, we still know very little about the underlying regulatory mechanisms involved.

A recent article by Lister et al. [7] has taken a step toward the better understanding of the dynamics and cell-type specificity of DNA methylation in the brain. The authors carried out a genome-wide mapping of cytosine methylation (by methylated cytosine sequencing (MethylC-seq)) and hydroxymethylation (by Tet-assisted bisulfite sequencing (TAB-seq)) at single-base resolution during the postnatal development of the mouse and human frontal cortex. In the mammalian nervous system, neural stem cells differentiate not only into neurons but also into glial cells (such as astrocytes and oligodendrocytes) that support neuronal activity. To account for possible cell-type-specific variations, the authors also generated epigenetic maps in separate populations of neurons and glial cells isolated by fluorescence-activated cell sorting. This technical tour de force provides an integrated view of the dynamic epigenome during mammalian brain development at an unprecedented resolution. These data confirm some previous observations by others and also reveal novel and unexpected findings that provide insights into potential pivotal functions.

**Accumulation of non-CG methylation and CG hydroxymethylation in adult neurons**

Lister et al. [7] first showed that while global CG methylation (mCG) levels are stable during brain development, the transition from the fetal to the adult cortex is characterized by a striking accumulation of DNA methylation in a non-CG context (that is, mCH, where H = A, C or T). mCH occurs mostly in CA sequences and rapidly accumulates during childhood concomitantly...
with synaptogenesis. In mice, this coincides with the transient upregulation of Dnmt3A, suggesting a role of this methyltransferase in establishing non-CG methylation.

In keeping with previous reports [8], Lister et al. [7] also demonstrated that the cortex acquires 5hmC during postnatal development; 5hmC occurs exclusively in the CG context, indicating that mCH is not susceptible to TET-mediated oxidation in the brain. They identified a small number of megabase-sized regions that are curiously refractory to the acquisition of mCH and 5hmC in the adult cortex, even though they have high levels of mCG. These regions, termed 'mCH deserts,' are enriched for large gene clusters and show signs of lower chromatin accessibility. By profiling isolated neurons and glial cells, Lister et al. then showed that mCH accumulates specifically in mature neurons, whereas glial cells retain low levels that resemble those of the fetal brain. In the cortical neurons of adult humans, mCH reaches levels that have never been observed in other mammalian cells and becomes the dominant form of methylation, accounting for approximately 53% of methylated cytosine residues, whereas mCG represents approximately 47%. In addition, the mCH positions throughout the genome are highly conserved between unrelated individuals, suggesting that mCH is the product of a controlled process with possible biological roles. Alternatively, the precise positioning of mCH might reflect the physical constraints of chromatin rather than a conserved, regulated function.

CG and non-CG methylation patterns discriminate functional gene categories

Are patterns of CG and CH methylation associated with specific gene functions? To address this question, Lister et al. [7] performed an in-depth analysis of methylation levels in 1 kb bins within and around each gene. One remarkable feature is that cell-type-specific variations in methylation are precisely localized to the bodies of genes. Highly expressed genes involved in neuronal and synaptic function do not acquire intragenic mCH in mature neurons, and are marked by reduced intragenic mCG in neurons compared with glial cells. Conversely, genes associated with glial functions gain high intragenic mCH in mature neurons and are marked by reduced intragenic mCG in glial cells compared with neurons.

The inverse relationship observed between intragenic mCH and mRNA abundance suggests a possible role in transcriptional repression. This is surprising because studies in other cell types have uncovered a positive correlation between intragenic DNA methylation and transcription, which suggests that distinct mechanisms of epigenetic regulation may operate in the brain compared with other tissues. Notable differences in gene body methylation were also evident on the X chromosome; Lister et al. [7] identified a subset of genes, in both humans and mice, with greater intragenic mCH levels (but not mCG levels) in female neurons compared with males. Remarkably, these genes correspond to those that escape X-inactivation in females, suggesting that their epigenetic signature could serve as a predictive feature. Indeed, on this basis, seven novel genes that escape X-inactivation were predicted.

CG hydroxymethylation marks sites of activation during brain development

Lister et al. [7] asked if 5hmC appears at specific genomic sites during postnatal brain development and if it is implicated in developmentally regulated demethylation. The base resolution analysis by TAB-seq revealed that 5hmC in the adult murine frontal cortex is depleted in transcription start sites but accumulates throughout the body of highly transcribed genes, where it inversely correlates with intragenic mCG levels. This confirms recent results obtained by other groups that used different mapping technologies to investigate mouse neuronal cell types [8,9]. 5hmC is also present at many DNase I hypersensitive sites and enhancer regions in the mouse brain.

Recently, the first single-base resolution map of 5hmC in embryonic stem cells revealed that this mark is enriched at active enhancers that undergo activation-coupled CG demethylation [10]. The work by Lister et al. [7] found that this is likely also to be the case in the brain in vivo: a set of enhancers were identified that undergo CG demethylation in the adult brain and are marked by 5hmC in the fetal brain, suggesting that 5hmC marks these poised enhancers for demethylation and activation later in development. To validate this model, Lister et al. [7] investigated CG methylation in the frontal cortex of Tet2-deficient mice and observed a small increase in DNA methylation at enhancers that normally undergo demethylation. The tiny magnitude of this hypermethylation could be explained by a functional redundancy with the other Tet enzymes.

In summary, Lister et al. [7] have shown that neurons undergo an extensive epigenetic reconfiguration during postnatal brain development and acquire a peculiar epigenetic marking of DNA characterized by modified cytosine residues in both CG and non-CG contexts. In contrast, glial cells maintain epigenetic profiles that closely resemble those of the fetal brain. The fact that many aspects of this epigenetic pattern are conserved between mouse and humans denotes functional importance, which opens stimulating perspectives for future research. Why do neurons acquire an epigenetic profile of DNA that is so different from other cell types? Is it related to the fact that neurons stop dividing? How do these epigenetic profiles influence gene expression states?
And is there additional epigenetic heterogeneity between neuron subtypes? We can now expect new answers from the study of mouse genetic models, as well as from the identification of proteins that bind to methylated and hydroxymethylated cytosine residues [9]. This will help to tackle the fascinating challenge of understanding how epigenetic marks regulate brain maturation, which in turn has major clinical applications for neurological disorders.

Abbreviations
SmC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; DNMT, DNA (cytosine-5) methyltransferase; MethylC-seq, methylated cytosine sequencing; MECP2, methyl-CpG-binding protein 2; TAB-seq, Tet-assisted bisulfite sequencing; TET, Ten-eleven translocation.

Competing interests
The authors declare that they have no competing interests.

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