⊚ MODES OF TRANSCRIPTIONAL REGULATION

Chromatin modifiers and remodellers: regulators of cellular differentiation

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Abstract | Cellular differentiation is, by definition, epigenetic. Genome-wide profiling of pluripotent cells and differentiated cells suggests global chromatin remodelling during differentiation, which results in a progressive transition from a fairly open chromatin configuration to a more compact state. Genetic studies in mouse models show major roles for a variety of histone modifiers and chromatin remodellers in key developmental transitions, such as the segregation of embryonic and extra-embryonic lineages in blastocyst stage embryos, the formation of the three germ layers during gastrulation and the differentiation of adult stem cells. Furthermore, rather than merely stabilizing the gene expression changes that are driven by developmental transcription factors, there is emerging evidence that chromatin regulators have multifaceted roles in cell fate decisions.

Cytosine methylation
The addition of a methyl group to the fifth carbon in cytosine, which predominantly occurs in the context of CpG dinucleotides ('p' refers to the phosphodiester bond that links a cytosine and a guanine). It is often referred to as DNA methylation and is a major form of DNA modification. Promoter methylation correlates with gene silencing.

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Nearly all cells of an organism share the same genome but show different phenotypes and carry out diverse functions. Individual cell types, which are characterized by distinct gene expression patterns, are generated during development and are then stably maintained. The chromatin state — the packaging of DNA with both histone and non-histone proteins — has marked effects on gene expression and is believed to contribute to the establishment and the maintenance of cell identities. Indeed, developmental transitions are accompanied by dynamic changes in chromatin states.

The assembly and the compaction of chromatin are regulated by multiple mechanisms, including DNA modifications (for example, cytosine methylation and cytosine hydroxymethylation), post-translational modifications (PTMs) of histones (for example, phosphorylation, acetylation, methylation and ubiquitylation), the incorporation of histone variants (for example, H2A.Z and H3.3), ATP-dependent chromatin remodelling and non-coding RNA (ncRNA)-mediated pathways. In recent years, substantial progress has been made in understanding the roles of histone modifications and chromatin remodelling in cellular differentiation, which is the focus of this Review. For perspectives of other chromatin regulators (such as DNA methylation and hydroxymethylation, histone variants and ncRNAs) in pluripotency, differentiation and development, we refer readers to other recent reviews[1–5].

PTMs of histones may either directly affect chromatin compaction and assembly or serve as binding sites for effector proteins, including other chromatin-modifying or chromatin-remodelling complexes, and ultimately influence transcription initiation and/or elongation. Most, if not all, histone PTMs are reversible. Many enzymes that are involved in their addition and removal have been identified. These include histone acetyltransferases (HATs; also known as lysine acetyltransferases) and histone deacetylases (HDACs; also known as lysine deacetylases); lysine methyltransferases (KMTs) and lysine demethylases (KDMs); and ubiquitylation enzymes (that is, E1, E2 and E3 enzymes) and deubiquitylases (DUBs). These enzymes often exist in multisubunit complexes and modify specific residues either on the amino-terminal tails or within the globular domains of core histones (H2A, H2B, H3 and H4). For example, in the two repressive Polycomb group (PcG) protein complexes, Polycomb repressive complex 1 (PRC1) contains either ring finger protein 1A (RING1A) or RING1B, both of which catalyse the monoubiquitylation of histone H2A at lysine 119 (H2AK119ub1), and PRC2 contains enhancer of zeste 2 (EZH2), which catalyses the trimethylation of H3K27 (H3K27me3). Additionally, some Trithorax group protein complexes contain the mixed-lineage leukaemia (MLL) family of KMTs that catalyse the formation of the transcriptionally activating H3K4me3 mark. Beyond PTMs of histones, chromatin compaction is also affected by ATP-dependent chromatin-remodelling complexes that use energy from ATP hydrolysis to exchange histones and to reposition or evict nucleosomes. Approximately 30 genes that encode the ATPase subunits have been identified in mammals. On the basis of the sequence and the structure

Cytosine hydroxymethylation
A form of DNA modification that is generated by the oxidation of 5-methylcytosine, which is mediated by the TET family of hydroxylases. 5-hydroxymethylcytosine is an intermediate in DNA demethylation and may also be a stable epigenetic mark.

Non-coding RNA
(ncRNA). A functional RNA molecule that is not translated into proteins and that can regulate gene expression at various levels, such as transcription, splicing, mRNA stability and translation. ncRNAs include small ncRNAs (for example, microRNAs and siRNAs) and long ncRNAs (for example, X inactive specific transcript (*XIST*) and HOXA transcript antisense RNA (*HOTAIR*)).

Pluripotency
The ability of a cell to differentiate into all three germ layers (that is, the endoderm, mesoderm and ectoderm) and to give rise to all fetal or adult cell types (for example, cells of the inner cell mass of blastocyst stage embryos are pluripotent).

Polycomb group
(PcG). A family of chromatin regulatory proteins that are typically involved in repressing gene expression, partly through the trimethylation of histone H3 lysine 27 and the monoubiquitylation of histone H2A lysine 119.

Trithorax group
A family of chromatin regulatory proteins that typically activate gene expression through the trimethylation of histone H3 lysine 4 and/or ATP-dependent chromatin remodelling.

Chromatin dynamics
Changes in chromatin structure, composition and positioning.

Inner cell mass
(ICM). A group of cells inside a mammalian blastocyst that gives rise to the embryo.

of these ATPases, chromatin-remodelling complexes are divided into four main families: SWI/SNF, ISWI, chromodomain-helicase DNA-binding protein (CHD) and INO80 complexes[6]. Many histone modifiers and chromatin remodellers have been implicated in stem cell pluripotency, cellular differentiation and development.

In this Review, we focus on chromatin dynamics in mammalian systems. We first describe chromatin states in stem cells and their alterations during differentiation, and we highlight findings from recent genome-wide profiling studies. This information provides important clues both to the functions of chromatin regulators and to the overall organization of chromatin in pluripotent cells compared with that in differentiated cells. We then review recent discoveries from genetic studies in mouse models to highlight the importance of various chromatin modifiers and remodellers in key developmental transitions. Finally, we discuss emerging evidence of new roles for chromatin regulators in cell fate decisions.

Epigenetic landscape in ES cells

Stem cells usually exist in small numbers in developing embryos and in somatic tissues, which makes it difficult to study the molecular mechanisms that govern stem cell self-renewal and differentiation *in vivo*. Embryonic stem (ES) cells, which are derived from the inner cell mass (ICM) of blastocysts, can be maintained and expanded indefinitely in culture while retaining their differentiation potential. Thus, ES cells are widely used as an experimental system for investigating the epigenetic regulation of stem cells.

Open chromatin of ES cells. A unique network of transcription factors, including the core pluripotency factors OCT4 (also known as POU5F1), sex-determining region Y-box 2 (SOX2) and homeobox protein NANOG, is involved in the establishment and the maintenance of ES cell pluripotency. ES cells also have distinctive chromatin features. Electron microscopy indicates that undifferentiated human ES cells have less heterochromatin than differentiated cells[7]. Staining of H3K9me3 and heterochromatin protein 1 (HP1), as well as fluorescence *in situ* hybridization (FISH) analysis of the major satellite DNA repeats, also suggests that constitutive heterochromatin is less condensed in undifferentiated ES cells than in differentiated cells. Consistently, major architectural chromatin proteins, such as HP1 and linker histones, are hyperdynamic and bind loosely to chromatin in these cells[8]. Genome-wide maps of epigenetic modifications from both mouse and human ES cells also revealed widespread active chromatin domains, which are characterized by enrichment of both histone acetylation and H3K4me3, and by hypomethylation of DNA[9–12]. The hyperactivity of the ES cell genome leads to widespread expression of both coding and non-coding elements[13]. Collectively, these findings indicate that ES cells have a globally 'open' and dynamic chromatin state (FIG. 1a).

Despite a highly active transcriptome, repression of lineage-specific genes is essential for maintaining ES cell pluripotency. A subset of developmental genes seem to be enriched with 'bivalent' domains, which contain both repressive H3K27me3 and activating H3K4me3 marks, in ES cells[9,14–17]. Recent evidence suggests that the two marks do not coexist on the same H3 tail but can occur on opposite H3 tails in the same nucleosome[18]. Bivalent domains have also been identified in pluripotent ICM and epiblast cells of early mouse embryos, multipotent haematopoietic stem cells (HSCs) and zebrafish blastomeres[19–23]. After differentiation of ES cells, most bivalent genes lose one of the marks and become monovalent[15]. These findings led to the notion that bivalent domains keep key developmental genes in a silent but 'poised' state in pluripotent cells. However, this hypothesis has been a topic of debate. Bivalency does not seem to be a universal feature of pluripotent and multipotent cells. For example, analyses of developing *Xenopus laevis* and *Drosophila melanogaster* embryos and of mouse hair follicle stem cells (HFSCs) identified few bivalent domains[24–26]. In addition, bivalency is not unique to pluripotent cells, as bivalent domains are also present, albeit in smaller numbers, in differentiated cells such as T lymphocytes, mouse embryonic fibroblasts and neurons[15,27,28]. Furthermore, the number of bivalent domains in ES cells could have been overestimated owing to the heterogeneity of histone marks in populations of cultured ES cells. ES cells that are grown in standard medium (which contains serum factors) include subpopulations of differentiating cells and show heterogeneity in both morphology and expression of pluripotency factors[29,30]. One study[31] analysed fractionated human ES cell subpopulations and found that some lineage-specific genes that are marked by bivalent domains according to bulk assays on unfractionated cells are actually monovalent (that is, they contain either H3K4me3 or H3K27me3) in distinct cell populations. Mouse ES cells can be maintained in a naive state in the absence of serum by using a defined medium, which is known as 2i medium, that contains inhibitors of mitogen-activated protein kinase kinase (MEK) and glycogen synthase kinase 3 (GSK3)[32]. Recently, one study[33] showed that mouse ES cells that are grown in 2i medium, compared with those grown in serum-containing medium, show highly similar H3K4me3 profiles but substantially reduced prevalence of H3K27me3 at promoters, many fewer bivalent domains and lower, rather than higher, levels of expression of lineage-specific genes. Thus, a large proportion of bivalent domains in ES cells that are cultured in serum are due to the acquisition of H3K27me3 at promoters (FIG. 1a), which further calls into question the importance of bivalency in naive pluripotent cells.

Chromatin dynamics during differentiation. ES cell differentiation is accompanied by global chromatin remodelling, which results in a progressive transition from the open chromatin configuration described above to a more compact and repressive state. Microscopically, heterochromatin foci become more condensed and more abundant in differentiated cells than in undifferentiated cells, which correlates with less dynamic exchange of chromatin proteins[6,7]. A genome-wide analysis of H3K9me2 identified large organized chromatin lysine 9 modifications, which generally occur in
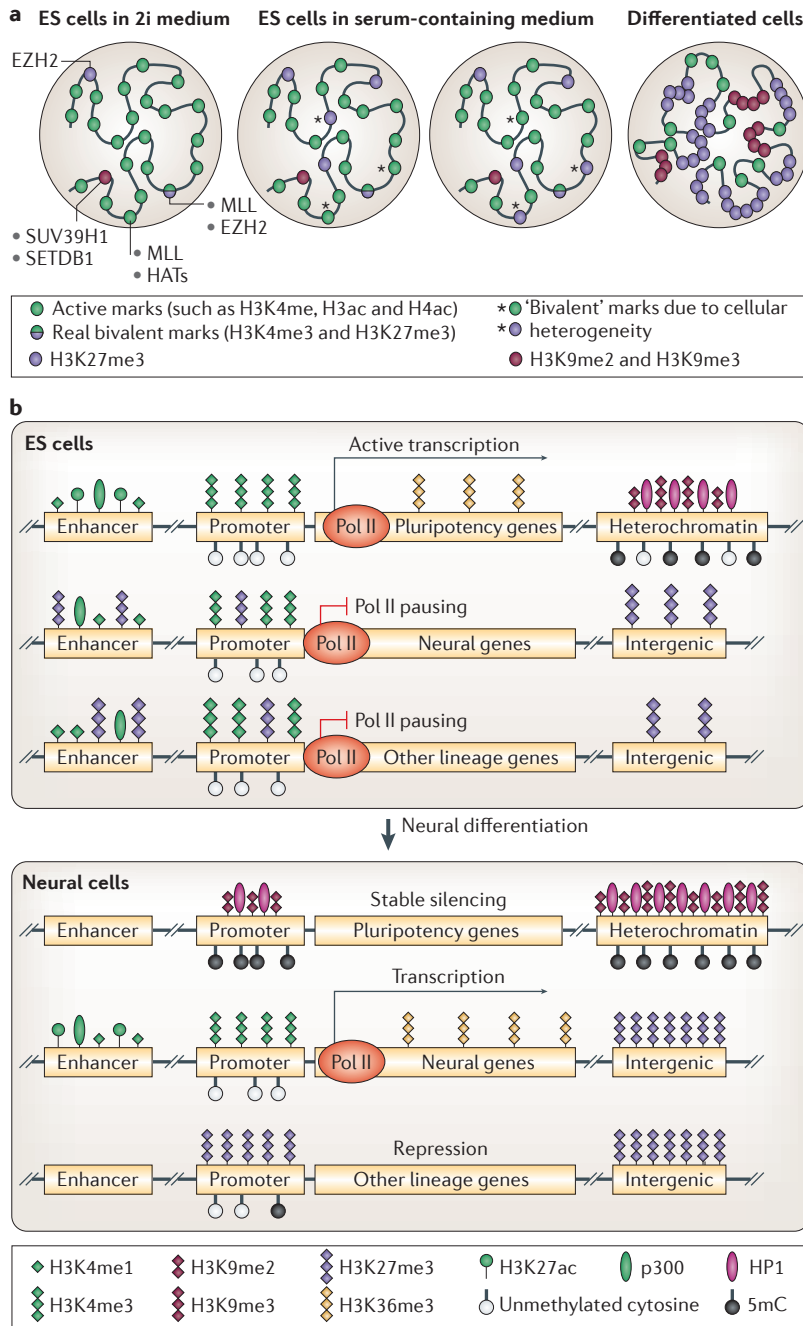
Figure 1 | **Chromatin states in pluripotent and differentiated cells. a** | Embryonic stem (ES) cells have a globally 'open' chromatin state, which is characterized by the enrichment of active histone marks such as histone acetylation (ac) and histone H3 lysine 4 methylation (H3K4me), whereas differentiated cells have a more compact chromatin state, which is characterized by expanded domains of repressive histone marks such as H3K27me3, H3K9me2 and H3K9me3. ES cells that are cultured in 2i medium are highly similar to the naive pluripotent cells in the ICM of blastocysts, and ES cells that are cultured in serum are more heterogeneous. Some H3K4me3 and H3K27me3 'bivalent' marks may reflect cellular heterogeneity, especially when ES cells are cultured in serum. **b** | Major chromatin features in different genomic regions are shown. In ES cells, the enhancers of both pluripotency genes and developmental lineage-commitment genes are enriched with H3K4me1 and histone acetyltransferase p300. The presence of H3K27ac makes enhancers of pluripotency genes active, whereas the lack of H3K27ac and the enrichment of H3K27me3 keep enhancers of developmental lineage-commitment genes in a 'poised' state. The promoters of pluripotency genes and lineage-commitment genes are also believed to be active and poised, respectively. Transcriptional elongation is prevented at lineage-commitment genes owing to promoter-proximal RNA polymerase II (Pol II) pausing. Upon differentiation towards a specific lineage (for example, the neural lineage), lineage-specific genes acquire active marks at both enhancer and promoter regions, and Pol II pausing is released to allow productive elongation. Genes of other lineages lose enhancer marks and gain H3K27me3 at promoters, which results in repression. Pluripotency genes gain H3K9 methylation and DNA methylation, and become stably silenced. During differentiation, heterochromatic regions — characterized by H3K9me2 and H3K9me3, heterochromatin protein 1 (HP1) binding and DNA methylation (that is, the formation of 5-methylcytosine (5mC) at CpG sites) — are expanded and become more condensed. H3K27me3 in both intergenic regions and repressed genes also expands to large domains. EZH2, enhancer of zeste 2; HAT, histone acetyltransferase; MLL, mixed-lineage leukaemia; SETDB1, SET domain, bifurcated 1; SUV39H1, histone lysine N-methyltransferase SUV39H1.

Blastocysts
Early stage embryos that have undergone the first cell lineage specification, which results in two primary cell types: cells of the inner cell mass and the trophoblasts.

Heterochromatin
Highly condensed chromatin that is transcriptionally inactive.

gene-poor facultative heterochromatin. These domains show significant increases in both genome coverage (4% versus 31%) and average size (43 kb versus 93 kb) as undifferentiated mouse ES cells progress through differentiation *in vitro*[34]. H3K27me3 also progresses from focal distributions in ES cells to expanded domains over silent genes and intergenic regions in differentiated cells[35,36] (FIG. 1a). Notwithstanding the evidence and the prevailing view of global chromatin remodelling, there are reports that suggest a greater role for local chromatin changes during cellular differentiation. For example, one study[37] showed that, during neuronal differentiation of ES cells, H3K9me2 shows no global increase but instead discrete local changes, particularly in genic regions.

During differentiation, ES cells silence pluripotency genes and gain the phenotypes of distinct differentiated cells by activating lineage-specific genes and by repressing lineage-inappropriate genes. Several groups recently carried out genome-wide transcriptional and epigenetic profiling of cells that are derived from directed differentiation of ES cells which represent both various lineages and defined differentiation stages[38–45]. From these studies, a global picture of epigenetic and gene expression alterations during differentiation is beginning to emerge (FIG. 1b). For example, active genes generally contain H3K4me3 at their promoters, and H3K4me1, H3K4me2 and H3K27 acetylation (H3K27ac) at their enhancers. Repressed loci are enriched with H3K27me3

and/or DNA methylation, which seem to repress distinct loci. One study[45] reported that promoters of developmental regulators that are active in early developmental stages tend to be CG rich and mainly use H3K27me3 upon silencing in non-expressing lineages; by contrast, somatic-tissue-specific promoters, which are active later in development, are generally CG poor and often show high levels of DNA methylation upon subsequent repression. At putative distal regulatory elements, one study[44] found lineage-specific transitions from high DNA methylation to H3K4me1 or H3K27me3. These alterations occur at many sites that do not seem to change gene expression during early stages of differentiation, which raises the possibility that these changes are epigenetic priming events that facilitate gene expression at later stages. Another interesting finding from the genome-wide studies is that some genes that have similar expression profiles during differentiation show considerable variations in chromatin states. For example, during cardiac differentiation, genes that are associated with metabolic function share a similar chromatin pattern, whereas those involved in contractile function and sarcomere structure have a distinct pattern, even though these two groups of genes have similar spatiotemporal expression profiles[40,41]. These findings imply that epigenetic regulation ensures coordinated expression of functionally related genes during differentiation. In summary, information about the chromatin state in ES cells and chromatin dynamics during ES cell differentiation could shed light on the functions of chromatin regulators in stem cell pluripotency and in cellular differentiation.

Chromatin states in adult stem cells
Many adult tissues harbour multipotent stem cells, which have the ability for life-long self-renewal and the ability to differentiate into various tissue-specific cell types. Adult stem cells are crucial for tissue homeostasis and regeneration. For example, HSCs give rise to all the blood cell types and are responsible for the constant renewal of blood, and neural stem cells (NSCs) produce the three primary cell types in the central nervous system — neurons, astrocytes and oligodendrocytes — and are the source of adult neurogenesis.

Although the scarcity of stem cells in most tissues remains a major challenge in studying adult stem cells, several groups were able to isolate sufficient quantities of adult stem cells from tissues to carry out transcriptional and epigenetic profiling studies. Results from the limited number of studies that are currently available support the notion that the chromatin states of adult stem cells are intermediate between those of pluripotent cells and terminally differentiated cells. For example, although the chromatin of adult stem cells is globally less open than that of ES cells, a common set of 'stemness' genes — including genes that encode regulators of chromatin, transcription, cell cycle and survival — is marked by H3K4me3 and is active in both HFSCs and ES cells[25]. In HSCs, H3K4me3 is more prevalent than differentiated progeny cells, and enhancers of differentiation genes are marked by the monomethylation of H3K4, H3K9

and H3K27, which is probably involved in the maintenance of the activation potential that is required for differentiation[21].

In the skin, HFSCs drive synchronized cycles of hair follicle growth (that is, anagen), destruction (that is, catagen) and rest (that is, telogen). One study[26] profiled global mRNA and histone methylation marks in quiescent (that is, telogenic) and activated (that is, anagenic) HFSCs and their committed, transit amplifying cell (TAC) progeny. During the transition from a quiescent state to an active, proliferative state, HFSCs show induction of cell cycle regulators without global alterations in mRNA and histone modification patterns. However, the transition from HFSCs to TACs involves substantial changes in transcriptional and chromatin profiles, including PcG-mediated repression of HFSC genes and derepression of PcG-silenced TAC regulators[25]. Similarly, comparisons of histone modification maps and gene expression profiles of human CD133+ HSCs and CD36+ erythrocyte precursor cells revealed that epigenetic changes correlate with changes in gene expression during erythrocyte differentiation. Specifically, the level of gene expression positively correlates with H3K4me3, H3K4me1, H3K9me1, H3K36me3 and H4K20me1 levels, and negatively correlates with H3K9me3 and H3K27me3 levels[22]. Mesenchymal stem cells (MSCs) are present in several tissues — including the bone marrow, umbilical cord and adipose tissue — and can be expanded in culture and induced to differentiate into various lineages, such as the osteoblasts, chondrocytes and adipocytes. Recent reports indicate that, when MSCs are induced to differentiate, histone modifications show dynamic changes, whereas promoter DNA methylation shows only modest changes that do not correlate significantly with changes in gene expression[46–48]. Comparisons of the DNA methylation maps of stem cells, progenitor cells and terminally differentiated cells of the blood and skin lineages also suggest that in vivo differentiation of HSCs and HFSCs is associated with fairly small changes in DNA methylation[49]. Previous studies revealed that, during multistep differentiation of mouse ES cells, most DNA methylation changes occur at the initial step of differentiation[11,28]. Therefore, it is likely that promoter DNA methylation patterns have been mostly established by the adult stem cell stage and that histone modifications have important roles in subsequent differentiation.

Genetic studies in mouse models
Development from a zygote to an organism is a complex process that involves multiple key cell fate decisions. During mammalian development, the zygote and the cells of early cleavage stage embryos are totipotent, as they can give rise to all embryonic and extra-embryonic tissues. The first cell lineage specification event occurs before implantation and results in the segregation of cells of the trophoblast (that is, the outer layer) and the ICM at the blastocyst stage. Following implantation, the trophoblast develops into placental tissues, and the pluripotent ICM develops into the epiblast, which differentiates to form the three germ layers — the ectoderm, mesoderm and endoderm — during gastrulation. The germ layers,
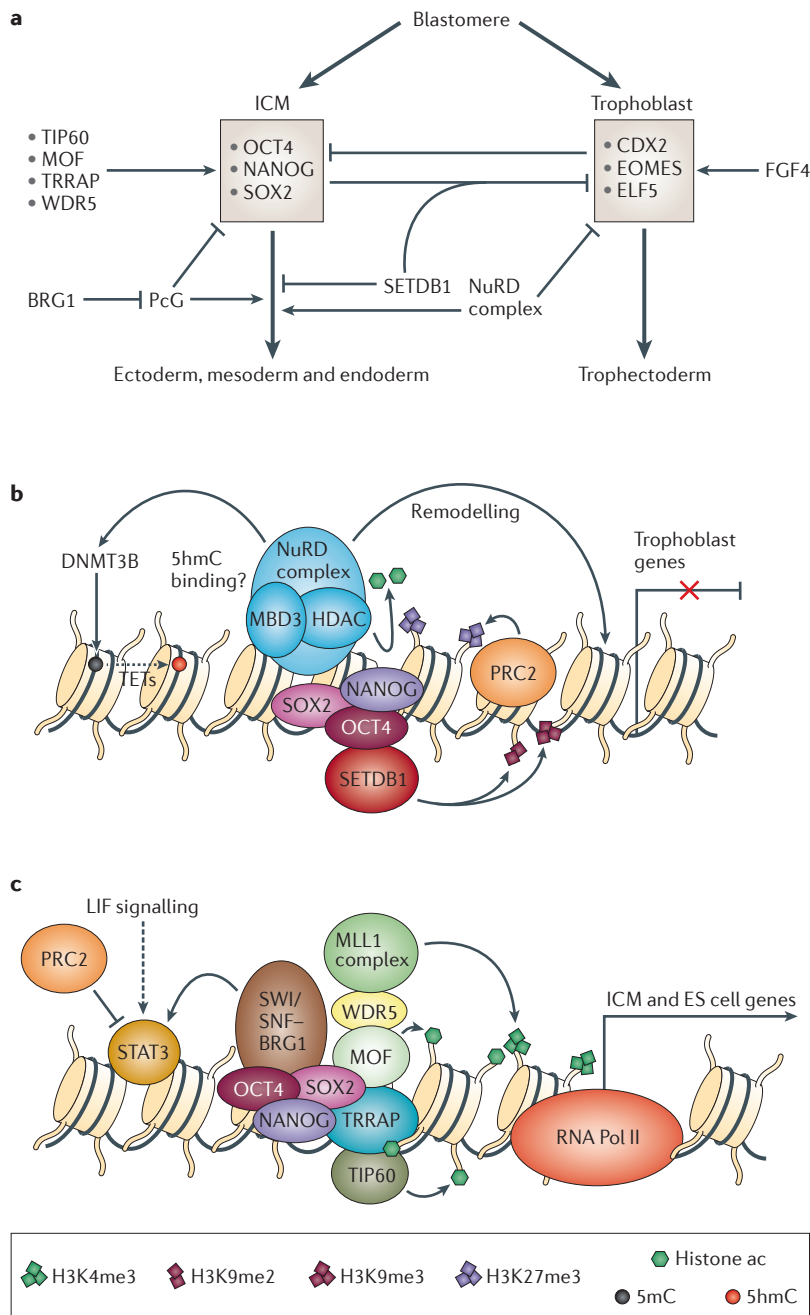
Figure 2 | **Chromatin regulators that are involved in the segregation of embryonic and extra-embryonic lineages during pre-implantation development. a** | The transcription factors that specify cells of the inner cell mass (ICM) and the trophoblast reciprocally antagonize each other. Chromatin regulators maintain the identity of cells of the ICM by repressing the trophectoderm transcriptional programme, preventing differentiation towards the three germ layers, promoting the expression of pluripotency factors and functioning as either co-regulators or effectors of pluripotency factors. Chromatin regulators that promote trophoblast differentiation are less well understood. **b** | In cells of the ICM, SETDB1 (SET domain, bifurcated 1) functions as a co-repressor of OCT4 to repress trophoblast genes by depositing histone H3 lysine 9 dimethylation (H3K9me2) and H3K9me3 marks. The nucleosome remodelling and deacetylation (NuRD) complex is also involved in the repression of trophoblast genes, possibly by multiple mechanisms. The NuRD complex has chromatin-remodelling activity that alters DNA–histone interactions. Histone deacetylase 1 (HDAC1) and HDAC2 are components of the NuRD complex, and the deacetylation of H3K27 has been shown to facilitate Polycomb repressive complex 2 (PRC2) binding and H3K27 methylation. The NuRD complex may also promote DNA methylation by inducing the expression of DNA methyltransferase 3B (DNMT3B). Recent evidence suggests that the methyl CpG-binding domain protein 3 (MBD3) subunit of the NuRD complex can bind to 5-hydroxymethylcytosine (5hmC). **c** | In cells of the ICM, pluripotency factors — such as OCT4, homeobox protein NANOG and sex-determining region Y-box 2 (SOX2) — recruit histone-modifying enzymes (for example, TIP60, MOF and mixed-lineage leukaemia (MLL) complexes) and chromatin-remodelling complexes (for example, SWI/SNF–BRG1) to ICM genes (including *Oct4*, *Nanog* and *Sox2* themselves) and their targets to create 'open' chromatin states. One of the effects of the SWI/SNF–BRG1 complex is to maintain chromatin accessibility at STAT3 (signal transducer and activator of transcription 3)-binding targets by opposing Polycomb group (PcG)-mediated repression, thus enhancing leukaemia inhibitory factor (LIF) signalling. 5mC, 5-methylcytosine; ac, acetylation; CDX2, caudal type homeobox 2; ELF5, E74-like factor 5 (ETS domain transcription factor); EOMES, eomesodermin homologue; ES, embryonic stem; FGF4, fibroblast growth factor 4; TET, methylcytosine dioxygenase; TRRAP, transformation/transcription domain-associated protein; WDR5, WD repeat domain 5.

Gastrulation
A phase of early embryonic development during which the three germ layers are formed.

which are multipotent, will give rise to specific tissues and organs in the developing embryo. Genetic studies in mouse models show major roles for a variety of chromatin modifiers and remodellers in key developmental transitions.

Pre-implantation development and ES cell identity.
Proper segregation of the ICM and trophoblast lineages at the blastocyst stage requires the transcription factors OCT4 (which determines commitment to the embryonic lineage) and caudal type homeobox 2 (CDX2, which specifies the trophoblast lineage). Reciprocal inhibition between the OCT4 and CDX2 transcription networks reinforces ICM-specific and trophoblast-specific expression patterns[50–52] (FIG. 2a). SETDB1 (SET domain, bifurcated 1; also known as ESET and KMT1E) — a histone methyltransferase that represses gene expression by catalysing the formation of H3K9me2 and H3K9me3 — seems to function as a co-repressor for OCT4 in this context. In mouse embryos, zygotic *Setdb1* expression begins at the blastocyst stage, specifically in cells of the ICM. A null mutation of *Setdb1* results in pre-implantation lethality and prevents both the proper development of the ICM and the establishment of ES cell lines[53]. Depletion of *Setdb1* in ES cells, either by short hairpin RNA-mediated knockdown or by genetic ablation, induces differentiation, particularly towards the trophoblast lineage[54–57]. The phenotypes of *Setdb1*-deficient embryos and ES cells are

similar to those of *Oct4* mutants[50]. Molecular analyses revealed that SETDB1 and OCT4 physically interact[55,56]. OCT4 seems to recruit SETDB1 for repression of genes that encode developmental regulators in cells of the ICM, especially those involved in trophoblast differentiation, such as *Cdx2* (REFS 54–57) (FIG. 2a,b).

The nucleosome remodelling and deacetylation (NuRD) co-repressor complex also has a role in maintaining the barrier between the embryonic and the trophectodermal cell fates. Deletion of the methyl CpG-binding domain protein 3 gene (*Mbd3*), which encodes a core component of the NuRD complex, results in peri-implantation lethality, in which the ICM fails to develop to a mature epiblast[58]. *Mbd3*-deficient ES cells are viable and can self-renew, but they show inappropriate expression of trophectoderm-specific genes such as E74-like factor 5 (*Elf5*) and eomesodermin homologue (*Eomes*). Although *Mbd3* deficiency alone is not sufficient to induce trophectoderm differentiation, *Mbd3*-deficient ES cells can be converted to trophoblast cells when cultured either in ES cell medium without leukaemia inhibitory factor (LIF) or in trophoblast stem cell medium[59–61]. These results suggest that the NuRD complex contributes to the repression of trophectoderm determinant genes, so that ES cells are not responsive to trophectoderm-inducing signals such as fibroblast growth factor 4 (FGF4) (FIG. 2a). The NuRD complex has also been shown to suppress the expression of pluripotency genes in ES cells and to promote lineage commitment[62]. The seemingly contrasting effects of the NuRD complex suggest a possible role for this complex in maintaining the balance between self-renewal and differentiation. These effects are probably mediated by complex and interconnected mechanisms, as the NuRD complex, in addition to its chromatin-remodelling and histone deacetylase activities, has been functionally linked to H3K27me3, DNA methylation and DNA hydroxymethylation[61,63,64] (FIG. 2b).

Components of several other enzyme complexes that are involved in histone modifications and ATP-dependent chromatin remodelling are essential for ICM survival and for ES cell self-renewal. TIP60 (also known as KAT5) and MOF (also known as KAT8), which are two members of the MYST family of HATs, as well as transformation/transcription domain-associated protein (TRRAP) — a common component of several HAT complexes — are required for pre-implantation development. Although mouse embryos that are deficient in either *Tip60* or *Mof* survive to the blastocyst stage, they die shortly afterwards, and these blastocysts fail to hatch and survive in cuture[65–67]. *Trrap*-null embryos show even more severe phenotypes, as 50% of blastocysts from *Trrap*^{+/−} intercrosses degenerate inside the zona pellucida, and *Trrap*^{−/−} blastocysts show severe growth retardation of the trophoblast layer and an absence of the ICM[68]. Conditional deletion of either *Mof* or *Trrap* in ES cells leads to a loss of self-renewal capability that is associated with alterations in histone acetylation and in chromatin structure[69,70], which is consistent with a previous RNA interference screen that identified *Trrap* and *Tip60* as regulators of ES cell identity[71]. ES cells that are deficient in either *Trrap* or *Mof* show a marked downregulation of pluripotency genes and an upregulation of specific differentiation markers of the three germ layers[69,70]. MOF, which catalyses the acetylation of H4K16, directly binds to pluripotency genes, including *Nanog*, *Oct4* and *Sox2*, and specifically regulates the NANOG transcriptional network[69]. WD repeat domain 5 (WDR5), which is a commonly shared component of the MOF and MLL complexes, also regulates ES cell self-renewal[72], although its role in mammalian development remains to be determined. Through WDR5, MOF may target MLL complexes and H3K4 methylation to pluripotency genes, which highlights the cooperation of various chromatin regulators in maintaining pluripotency[69] (FIG. 2a,c).

BRG1, which is the ATPase subunit of the SWI/SNF–BRG1 chromatin-remodelling complex, is present throughout pre-implantation development[73]. Maternal *Brg1* is required for zygotic genome activation at the two-cell stage[74], and zygotic *Brg1* is essential for the survival and the proliferation of cells of the ICM and the trophoblast[75]. SMARCB1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1; also known as SNF5, BAF47 and INI1) and SMARCC1 (also known as BAF155 and SRG3), which are two other components of the SWI/SNF complex, are also required for peri-implantation development[76,77]. Depletion of *Brg1* in ES cells results in a loss of self-renewal and induces differentiation[78,79]. A genome-wide analysis revealed that BRG1 extensively colocalizes with pluripotency factors in ES cells, which suggests that the SWI/SNF–BRG1 complex is an important component of the pluripotency network[78,80]. The cytokine LIF can support self-renewal of murine ES cells by activating STAT3 (signal transducer and activator of transcription 3). A recent study indicates that the SWI/SNF–BRG1 complex maintains chromatin accessibility at STAT3-binding targets by opposing PcG-mediated repression[81] (FIG. 2c).

In summary, the chromatin modifiers and remodellers described above regulate pre-implantation development and maintain the identity of ES cells and cells of the ICM by suppressing the trophectoderm transcriptional programme, preventing differentiation towards the three germ layers, promoting the expression of pluripotency factors and functioning as co-regulators or effectors of pluripotency factors (FIG. 2a).

Post-implantation development and ES cell differentiation. An important post-implantation developmental event is gastrulation, through which the three germ layers are formed and which results in the establishment of the basic body plan. Many chromatin regulators have been implicated in this process. Among the most extensively studied are PcG proteins. Embryos that lack EZH2, which is the histone methyltransferase catalytic subunit of PRC2, initiate but fail to complete gastrulation and die soon after implantation[82]. Deletion of the PRC2 core component EED or SUZ12 results in similar phenotypes[83,84]. RING1B — the histone ubiquitylation catalytic subunit of PRC1 — and some other PRC1 components (for example, RYBP (RING1 and YY1-binding protein) and L3MBTL2 (lethal(3)malignant brain tumour-like protein 2)) are also essential for both gastrulation and

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Nucleosome remodelling and deacetylation (NuRD) co-repressor complex
A multisubunit complex with both ATP-dependent chromatin remodelling and histone deacetylase activities. Its components include the chromodomain-helicase DNA-binding protein (CHD) family of ATPases Mi-2α/Mi-2β; histone deacetylase 1 (HDAC1) and HDAC2; metastasis-associated protein 1 (MTA1), MTA2 and MTA3; methyl CpG-binding protein 2 (MBD2) and MBD3; and histone-binding protein RBBP7 and RBBP4.

Leukaemia inhibitory factor
(LIF). An interleukin-6 class cytokine that is often added in mouse embryonic stem cell cultures to inhibit differentiation.

Trophoblast stem cell
A multipotent cell that can produce all trophoblast cell types in culture and *in vivo*.

Hatch
When a blastocyst bursts out of the protective zona pellucida.

Zona pellucida
A thick glycoprotein membrane that surrounds the plasma membrane of an oocyte.

early embryogenesis[85–87]. Consistent with the developmental phenotypes, ES cells that are deficient in PRC1 and/or PRC2 functions are capable of self-renewal, but they show inappropriate derepression of lineage-specific genes and differentiation defects[87–92]. PcG proteins are thus key components of a network that represses developmental genes during differentiation (FIG. 3a).

Pluripotency genes are rapidly repressed upon differentiation and remain stably silenced in differentiated cells. EHMT2 (also known as G9A) — a histone methyltransferase that catalyses mainly the formation of H3K9me2 in euchromatin — seems to be a key component of the machinery that silences pluripotency genes. Embryos that lack EHMT2 show prolonged expression of *Oct4* and *Nanog*, severe growth retardation and early lethality[93,94]. EHMT2-deficient ES cells show normal self-renewal, but fail to stably silence *Oct4* and show differentiation defects[93,95]. Inactivation of *Oct4* following embryo implantation is a multistep process that involves the direct inhibition of transcription, followed by the formation of local heterochromatin and *de novo* DNA methylation. EHMT2 is not required for the initial *Oct4* repression upon differentiation, but EHMT2-mediated H3K9 methylation is necessary for the subsequent formation of heterochromatin and *de novo* DNA methylation at the *Oct4* locus[95]. A recent study suggests that some signalling pathways influence differentiation by altering EHMT2 expression[94]. Although H3K9 methylation and the formation of heterochromatin may contribute to *de novo* DNA methylation, EHMT2 can also promote DNA methylation independently of its histone methyltransferase activity by recruiting the *de novo* DNA methyltransferases DNMT3A and DNMT3B[96–98]. DNA methylation profiling revealed that pluripotency genes and germline-specific genes are major targets of differentiation-coupled *de novo* DNA methylation[11,28], and genetic evidence indicates that DNMT3A and DNMT3B are required for methylation of the *Oct4*
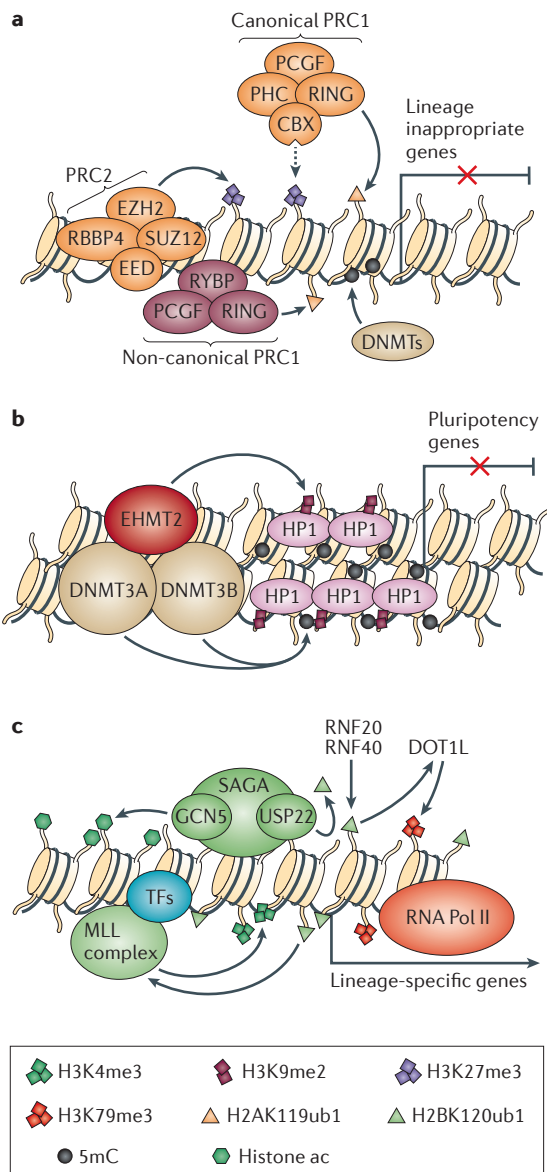

Figure 3 | **Chromatin regulators that are involved in gene regulation during post-implantation development and cellular differentiation.**
a | Polycomb group proteins have important roles in repressing developmental genes. Polycomb repressive complex 2 (PRC2) deposits the repressive histone H3 lysine 27 trimethylation (H3K27me3) marks, which creates binding sites for the canonical, chromobox homologue protein (CBX)-containing PRC1 complex (shown by the dashed arrow). The non-canonical, RYBP (RING1 and YY1-binding protein)-containing PRC1 complex binds to chromatin through H3K27me3-independent mechanisms. Ring finger protein 1A (RING1A) and RING1B, which are components of the PRC1 complexes, mediate the repressive histone H2A lysine 119 monoubiquitylation (H2AK119ub1). DNA methylation, which is mediated by DNA methyltransferases (DNMTs), is also important in repressing lineage-commitment genes, especially those with low CpG-content promoters. **b** | EHMT2 is crucial for silencing pluripotency genes in post-implantation embryos and in differentiated cells. EHMT2 deposits H3K9me2 marks, which induces the formation of heterochromatin by recruiting heterochromatin protein 1 (HP1). EHMT2 also recruits DNMT3A and DNMT3B, both of which initiate *de novo* DNA methylation. **c** | In differentiating cells, lineage-specific transcription factors (TFs) recruit chromatin-modifying complexes, such as the mixed-lineage leukaemia (MLL) complex and the SAGA complex, to lineage-specific genes to create 'open' chromatin states. The MLL complex deposits the active H3K4me3 marks. The SAGA complex has at least two enzymatic activities: histone acetylation (ac) by GCN5 and H2BK120 deubiquitylation by ubiquitin carboxyl-terminal hydrolase 22 (USP22). H2BK120ub1, which is deposited by RNF20 and RNF40, is preferentially enriched in the coding regions of lineage-specific genes, but not of pluripotency genes. H2BK120ub1 has been shown to promote both MLL-mediated H3K4 methylation and DOT1-like histone H3K79 methyltransferase (DOT1L)-mediated H3K79 methylation. 5mC, 5-methylcytosine; EZH2, enhancer of zeste 2; PCGF, Polycomb group ring finger proteins; PHC, Polyhomeotic homologue proteins; RBBP4, histone-binding protein RBBP4 (also known as RbAp48).

and *Nanog* promoters in differentiating ES cells and in post-implantation embryos[99]. Taken together, these findings suggest that histone methylation and DNA methylation function cooperatively to ensure complete and stable silencing of pluripotency genes (FIG. 3b).

During differentiation, proper activation of lineage-specific genes is as important as inactivation of both lineage-inappropriate genes and pluripotency genes. Multiple epigenetic factors that are associated with gene activation have been implicated in gene expression during cellular differentiation and embryogenesis (FIG. 3c). For example, embryos that lack GCN5 (also known as KAT2A) — a HAT that is part of the SAGA complex and a co-activator for multiple transcription factors — show both a loss of mesodermal tissues that is due to apoptosis and early embryonic lethality[100,101], and *Gcn5*-null ES cells form smaller embryoid bodies than wild-type ES cells[102].
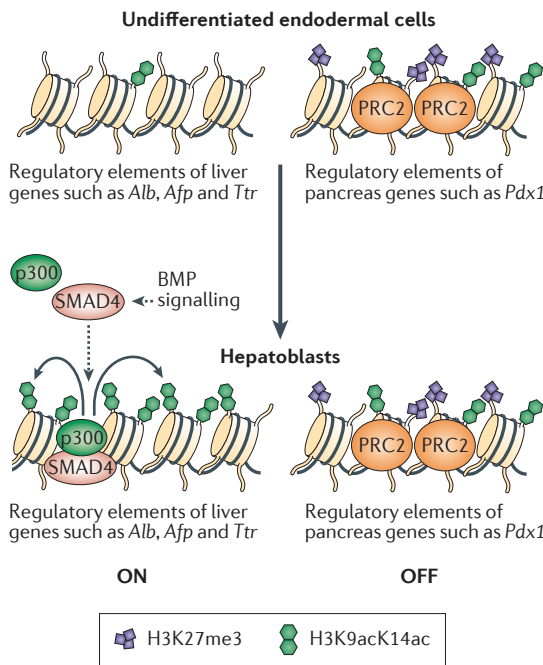

Figure 4 | Epigenetic 'pre-patterning' for lineage specification. In multipotent endodermal cells, regulatory elements of liver-specific genes and pancreas-specific genes are pre-patterned with distinct chromatin marks. Both the active histone H3 lysine 9 and lysine 14 acetylation (H3K9acK14ac) marks and the repressive H3K27 trimethylation (H3K27me3) marks are enriched in the regulatory elements of pancreas-specific genes (such as pancreatic and duodenal homeobox 1 (*Pdx1*)), but they are either low in abundance or undetectable in the regulatory elements of liver-specific genes (such as albumin (*Alb*), alpha fetoprotein (*Afp*) and transthyretin (*Ttr*)). In response to bone morphogenetic protein (BMP) signalling, SMAD4 recruits histone acetyltransferase p300 to the regulatory elements of liver-specific genes to stimulate histone acetylation and to induce hepatic specification. Polycomb repressive complex 2 (PRC2) maintains the level of H3K27me3 in the regulatory elements of pancreas-specific genes to prevent pancreas specification.

Interestingly, the loss of HAT activity of GCN5 is only partly responsible for these phenotypes, as embryos that are homozygous for mutations in the GCN5 catalytic site survive until mid-gestation, when they show severe neural tube closure defects[103]. Subsequent studies revealed that deletion of GCN5 affects the activity of a second enzyme in the SAGA complex — ubiquitin carboxyl-terminal hydrolase 22 (USP22) — which deubiquitylates histone H2B and non-histone proteins such as telomeric repeat-binding factor 1 (TRF1) and far upstream element-binding protein 1 (FUBP1)[104,105]. Mice that carry *Gcn5* null mutations have a more severe phenotype than those with *Gcn5* catalytic mutations, which probably reflects the combined loss of GCN5 and USP22 activities.

Several recent studies revealed that the level of H2BK120ub1 — a mark that is associated with highly transcribed genes — significantly increases upon differentiation of stem cells[106–108]. H2BK120ub1 is preferentially enriched in the coding regions of differentiation-related genes but not in pluripotency genes[108]. Inhibition of H2BK120 ubiquitylation, either by depletion of the RNF20–RNF40 E3 ligase complex or by ectopic expression of an H2B-K120R mutant, attenuates the upregulation of lineage-specific genes and impairs cellular differentiation[106–108]. H2BK120ub1 promotes H3K4 and H3K79 methylation, both of which are also associated with gene activation[109] (FIG. 3c).

Epigenetic modifiers in adult stem cell functions. Conditional knockout models, which circumvent the embryonic and postnatal lethality that often occurs in mice with germline gene deletions, indicate that many chromatin regulators that are involved in cell fate decisions during embryogenesis also have important roles in adult stem cell functions. For example, recent studies suggest that BRG1 has key roles in the proliferation and the differentiation of HFSCs, as well as in hair regeneration and epidermal repair[110]. However, some chromatin modifiers seem to be crucial in adult stem cells but not during embryonic development[111,112]. For example, mice that lack TET1 — a 5-methylcytosine (5mC) dioxygenase that converts 5mC to 5-hydroxymethylcytosine (5hmC) — are viable and fertile[113], but they show reduced self-renewal of NSCs in adult brain and impaired hippocampal neurogenesis[112].

Multifaceted roles of chromatin regulators
Although the classic view that transcription factors are major 'drivers' of differentiation and that chromatin modifiers are primarily responsible for stabilizing the differentiated states was important in the early stages of understanding the general roles of these two groups of proteins, this model has proven to be too simplistic to explain the complexity of the interactions between transcription factors and chromatin regulators. Recent evidence suggests that chromatin regulators are involved in priming transcriptional responses before cell fate decisions, in modulating gene expression during cellular differentiation and in transmitting epigenetic marks through cell divisions to maintain the identity of differentiated cells.

Box 1 | **Chromatin modifications and pioneer factor binding**

Pioneer factors are a special class of transcription factors that can access their DNA target sites in compact chromatin and that presumably bind to the genome before the binding of other factors. Multiple proteins have been shown to have the properties of pioneer factors. These include the forkhead box A (FOXA) factors, GATA-binding (GATA) factors, PU.1 and FOXD3 (REF. 118). Recent studies suggest that the reprogramming factors OCT4, sex-determining region Y-box 2 (SOX2) and Krüppel-like factor 4 (KLF4) are also pioneer factors. One study[131] showed that the vast majority of reprogramming factor-binding events that happen early in somatic cell reprogramming occur within closed chromatin. Pioneer factor binding is thought to impart competence for future gene expression by opening up the local chromatin and facilitating the subsequent recruitment of additional transcription factors and other regulatory proteins[118].

A defining feature of pioneer factors is their ability to access condensed chromatin without the aid of other factors, including chromatin modifiers and remodellers. However, pioneer factor binding can be either positively or negatively affected by special chromatin features. FOXA1 binding in breast cancer cells is facilitated by the absence of DNA methylation, nucleosome depletion and the presence of H3K4me1 and H3K4me2 marks[118]. Similarly, epigenetic 'pre-patterning' of the liver regulatory elements in undifferentiated endodermal cells correlates with FOXA, GATA4 and GATA6 binding[115]. In human somatic cells, megabase-scale chromatin domains that are enriched with H3K9me3 prevent the binding of OCT4, SOX2, KLF4 and MYC, and impede the reprogramming of these cells to pluripotency[131]. Chromatin modifications and pioneer factors are likely to function synergistically to impart competency for transcription.

Epigenetic pre-patterning for lineage specification. Transcription factors preferentially bind to open chromatin. Thus, epigenetic mechanisms may set the stage for lineage-specific transcription factors by creating and maintaining a permissive chromatin environment. Indeed, an emerging theme from recent studies is that epigenetic pre-patterning occurs before cell fate decisions. One study[114] differentiatied mouse ES cells towards the B cell lineage and investigated the epigenetic regulation of gene expression. The authors found that a *cis*-acting element in the immunoglobulin lambda-like polypeptide 1 (*Igll1*; also known as *Lambda 5*)–pre-B lymphocyte gene 1 (*VpreB1*) locus is marked by histone H3 acetylation and H3K4me2 at a discrete site in undifferentiated ES cells. The marked region expands during differentiation and becomes a localized centre for the recruitment of both transcription factors and RNA polymerase II before full activation of the *Igll1* and *VpreB1* genes at the pre-B cell stage[114]. Similar epigenetic pre-patterning has been shown in the fate choice of the liver and pancreas in the embryonic endoderm. One study[115] showed that regulatory elements of liver- and pancreas-specific genes have distinct chromatin patterns in undifferentiated endodermal cells. When the cells differentiate into hepatoblasts, H3K9ac and H3K14ac promote expression of hepatic genes, whereas H3K27me3 seems to repress the expression of pancreatic genes (FIG. 4).

The concept of transcriptional priming by chromatin changes is reinforced by recent studies of higher-order chromatin structure during induced 'dedifferentiation'. Circular chromosome conformation capture with high-throughput sequencing (4C–seq) reveals that, during somatic cell reprogramming into induced pluripotent stem cells (iPSCs), the establishment of long-range inter-chromosomal interactions with the *Oct4* and *Nanog* loci precedes transcriptional activation of these genes[116,117]. Recent genome-wide mapping studies suggest that epigenetic pre-patterning is a widespread phenomenon in cell fate decisions. For example, enhancers are usually pre-patterned by H3K4me1 and H3K4me2 marks, and by the histone variants H3.3 and H2A.Z before their target genes are activated. There is evidence that the presence of H3K4me1 and H3K4me2 marks facilitates the binding of 'pioneer factors' (REF. 118). Pioneer factor binding, albeit not sufficient for gene activation, opens up chromatin and imparts competence for transcription (BOX 1). Epigenetic pre-patterning may be important for the spatiotemporal regulation of gene expression during development.

Chromatin modifiers as co-regulators of transcription. Many histone-modifying enzymes are components of co-regulator complexes, which function cooperatively with transcription factors to modulate gene expression. In most cases, the core co-regulator complexes have no DNA-binding capability, and DNA-binding transcription factors, DNA methylation and ncRNAs have all been implicated in the recruitment of histone-modifying complexes. The heterogeneity in subunit composition of co-regulator complexes may also confer target selectivity and functional specificity. This idea is best supported by results from recent studies of the highly heterogeneous PRC1 complexes (BOX 2). Canonical chromobox homologue protein (CBX)-containing PRC1 complexes require the presence of H3K27me3 for their genomic localization, whereas non-canonical, RYBP-containing PRC1 complexes lack CBX proteins and show H3K27me3-independent recruitment and H2A ubiquitylation[119,120]. PRC1 complexes are also recruited to CpG islands by the H3K36-specific demethylase KDM2B[121,122]. Canonical PRC1 complexes that contain different CBX proteins also seem to have non-overlapping functions. CBX7 is the predominant CBX protein in ES cells and is required for pluripotency, whereas CBX2, CBX4 and CBX8 become upregulated upon differentiation and function in lineage commitment[123,124].

Co-regulators are often referred to as co-activators or co-repressors. For example, HAT-containing complexes (such as SAGA) usually function as co-activators, whereas HDAC-containing complexes (such as SIN3) are generally co-repressors. A surprising finding from recent genome-wide mapping studies is that some classic 'co-repressors' are associated not only with repressed genes but also with actively transcribed loci. For


Dedifferentiation
Conversion of a differentiated cell to a pluripotent or multipotent cell.

CpG islands
Genomic regions that contain a high content of CpG dinucleotides and that are found in many mammalian promoters.

Box 2 | **Heterogeneous compositions of mammalian PRC1 complexes**

In *Drosophila melanogaster*, the core Polycomb repressive complex 1 (PRC1) contains Polycomb (Pc), which is a chromo-domain-containing protein that binds to histone H3 trimethylated at lysine 27 (H3K27me3); Sex combs extra (Sce; also known as dRING), which is an E3 ligase that catalyses H2A monoubiquitylation; Posterior sex combs (Psc), which is a large protein that can induce chromatin compaction; and Polyhomeotic (Ph). Each core subunit has two or more homologues in mammals (see the figure). The homologues of Pc and Sce are Chromobox homologue proteins (CBX2, CBX4, CBX6, CBX7 and CBX8) and ring finger proteins (RING1A and RING1B), respectively. The homologues of Psc are Polycomb group ring finger proteins (PCGF1 (also known as NSPC1), PCGF2 (also known as MEL18), PCGF3, PCGF4 (also known as BMI1), PCGF5 and PCGF6 (also known as MBLR)); the homologues of Ph are Polyhomeotic homologue proteins (PHC1, PHC2 and PHC3). Combinatorial association of these different homologues gives rise to multiple canonical mammalian PRC1 complexes with distinct properties and functions. Moreover, recent studies have identified non-canonical PRC1 complexes, which contain RYBP (RING1 and YYI-binding protein) or a related protein YAF2 (YY1-associated factor 2) instead of CBX proteins[119,120]. Non-canonical PRC1 complexes have also been shown to associate with other proteins through individual subunits. Through PCGF1, non-canonical PRC1 complexes interact with BCL6 co-repressor (BCOR), lysine demethylase 2B (KDM2B) and S phase kinase-associated protein 1 (SKP1) to form the BCOR co-repressor complex[121,122]. Through PCGF6, components of non-canonical PRC1 complexes interact with lethal(3) malignant brain tumour-like 2 (L3MBTL2), E2F transcription factor 6 (E2F6), CBX3 (also known as HP1γ) and possibly the H3K9me2-specific methyltransferase EHMT2 to form the PRC1-like 4 (PRC1L4) complex[141].
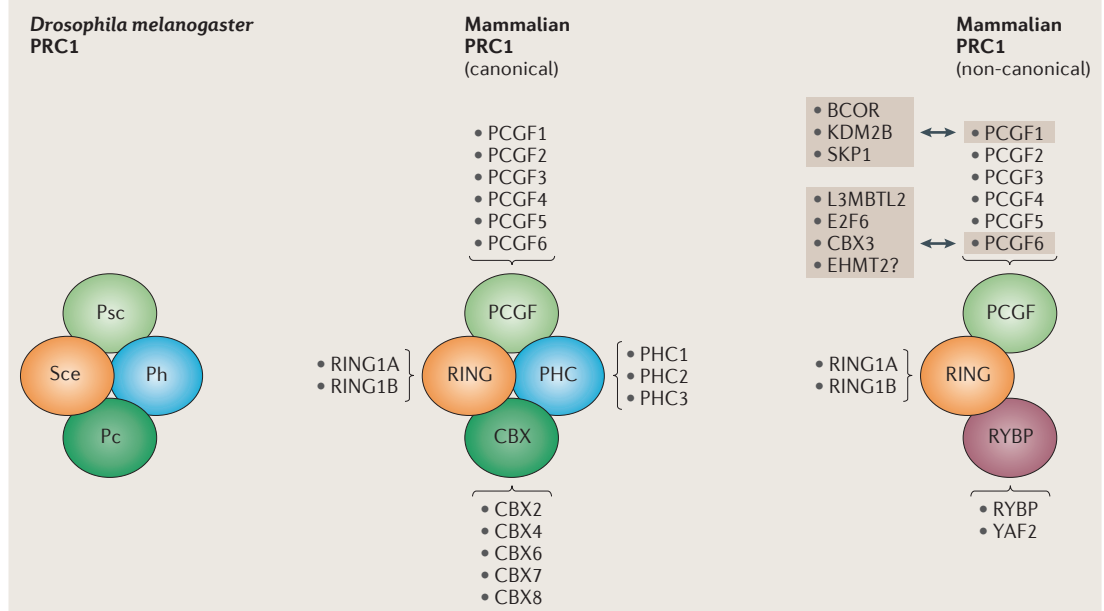
example, a chromatin immunoprecipitation followed by sequencing (ChIP–seq) analysis of multiple HATs and HDACs in human T cells revealed that all the HDACs examined are highly enriched in active genes and that only a minor proportion of these HDACs are associated with silent genes[125]. The yeast Rpd3S HDAC complex has also been shown to be recruited to transcribed chromatin to prevent cryptic initiation of transcription within the coding region[126]. The precise control of gene expression levels is crucial for cell fate determination, and co-repressors may have important roles in this 'fine-tuning' of gene expression.

Inheritance of chromatin modifications. Cellular identities, once established, are remarkably stable. Although cellular identities can be experimentally reprogrammed either by cell fusion or by forced expression of pluripotency-associated or lineage-specific factors, cellular reprogramming is a slow and inefficient process. Chromatin modifications, such as DNA methylation

and H3K9me3, are major barriers for the reprogramming of somatic cells into iPSCs, which highlights the importance of chromatin modifications in cellular memory[127]. Indeed, the efficiency of iPSC derivation can be increased by modulating chromatin regulators such as DNMTs, KMTs and chromatin remodellers[128–133]. For example, a recent study showed that, strikingly, depletion of MBD3 results in reprogramming efficiency of up to 100% within seven days[133]. Interestingly, compared with primary cells, cells that are grown as adherent cultures in the presence of serum tend to form marked large-scale H3K9me3 domains, which may hinder reprogramming[36].

A fundamental question to our understanding of long-term maintenance of cellular identity is how chromatin modifications are passed to daughter cells through cell divisions. It is widely accepted that symmetrical CpG methylation is faithfully maintained during DNA replication by a mechanism that involves semi-conservative segregation and template copying
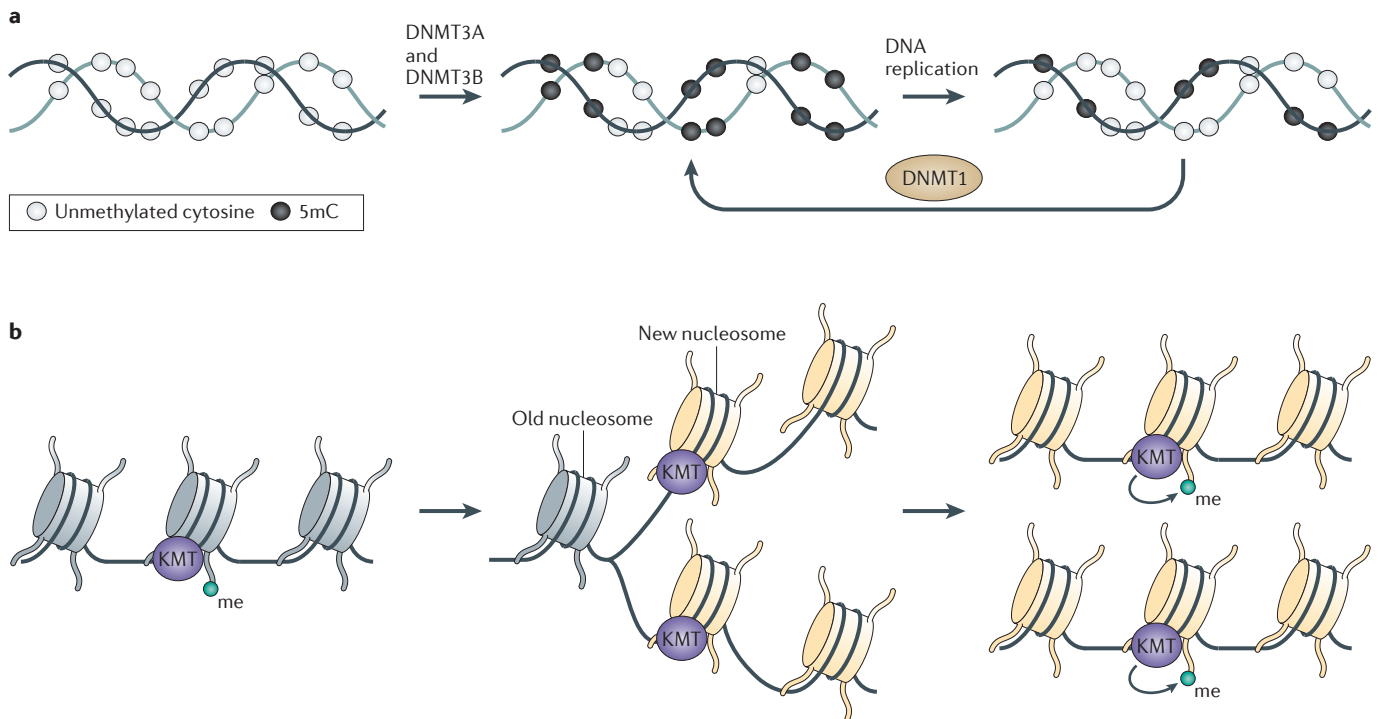
Figure 5 | **Inheritance of DNA methylation and histone methylation marks through DNA replication.**
a | Semi-conservative maintenance of symmetrical cytosine methylation (5mC) at CpG sites is shown. During early embryogenesis, DNA methylation patterns are established by the *de novo* DNA methyltransferases DNMT3A and DNMT3B. After each round of DNA replication, the maintenance DNA methyltransferase DNMT1 'copies' the 5mC patterns from the parental strand onto the daughter strand. **b** | The role of lysine methyltransferases (KMTs) in maintaining histone methylation (me) is shown. During DNA replication, methylated histones are replaced by unmodified histones, but KMTs remain associated with newly replicated DNA at specific loci. Following DNA replication, the enzymes methylate the newly incorporated histones to re-establish the methylation patterns.

(FIG. 5a). However, the mechanisms by which histone modifications are mitotically inherited are poorly understood. Several models have been proposed to explain the inheritance of histone methylation marks, which show fairly slow turnover and thus have the potential to be mitotically heritable[134]. Recent evidence suggests that, at least in some cases, histone-modifying enzymes, rather than the histone marks, persist through DNA replication. One study[135] showed that, in *D. melanogaster* embryos, H3K4me3 and H3K27me3 are replaced by non-methylated H3 following DNA replication, whereas the H3K4 methyltransferase Trithorax and the H3K27 methyltransferase Enhancer of zeste remain associated with newly replicated DNA. *In vitro* experiments also revealed a continuous association of PRC1 with replicating DNA[136]. These results support a model that histone methyltransferase complexes that are associated with nascent DNA re-establish histone methylation marks on newly assembled nucleosomes (FIG. 5b). It will be important to determine the generality and the importance of this model in epigenetic inheritance.

Conclusion
Recent technological advances have led to comprehensive epigenomic maps in pluripotent and differentiated cells. The results support the notion that differentiation is accompanied by dynamic changes in chromatin states, which implies important functions for chromatin regulators in cell fate decisions. Although a global picture of the chromatin states in pluripotent cells and their changes during differentiation is emerging, it is far from complete. Several prevalent histone modifications have been the focus of most published studies, and the majority of histone modifications have not been explored[137]. Moreover, various histone marks function collaboratively and coordinately in biological processes. An important area of future research is therefore to determine the 'meanings' of different combinations of histone modifications.

Most of the published genome-wide chromatin modification studies have compared undifferentiated ES cells with *in vitro* differentiated cells. Although ES cells can recapitulate many aspects of early embryogenesis, their epigenome is not identical to that of cells of the ICM and varies in different culture conditions. Additionally, ES cells from different species may represent different developmental stages. There is evidence that human 'ES cells' are actually more similar to mouse epiblast-derived stem cells (EpiSCs) than to mouse ES cells[138]. Furthermore, differentiation of ES cells, in most cases, produces heterogeneous cell populations. In the future, we expect that highly sensitive technologies, including single-cell assays, will be developed so that small numbers of stem cells or

Epiblast-derived stem cells
(EpiSCs). Pluripotent stem cells that are derived from the late epiblast layer of post-implantation embryos.

other types of cells that are isolated from animals and humans can be directly profiled.

Loss-of-function and gain-of-function studies are powerful approaches for investigating the roles of individual genes in biological processes. Genetic studies in mice and in other model organisms have clearly shown the importance of chromatin regulators in major developmental transitions. However, the developmental functions of many other chromatin regulators remain to be explored. It is worth noting that phenotypes of mutant animals may not be entirely attributable to chromatin defects. Most 'histone' modifiers probably also modify non-histone proteins, and loss-of-function and gain-of-function models could facilitate the identification of these non-histone substrates. A bottleneck is that, for many modifications, 'pan' antibodies that recognize diverse substrates that are marked by the modification are not readily available, and it is difficult to identify some PTMs by mass spectrometry. Another major challenge is to determine the biological functions of modifications on non-histone proteins. These modifications may be 'read' by protein domains that recognize such marks in histones and may be subject to regulatory crosstalk, in which different modifications regulate one another, as is observed in histones. PTM crosstalk can even occur between histone and non-histone proteins, which perhaps foreshadows the discovery of chromatin signalling cascades[139]. Genome-wide studies that compare wild-type and mutant cells will no doubt continue to provide new clues to the full range of histone modifier functions.

Consistent with their fundamental role in differentiation, many chromatin modifiers and remodellers have been implicated in various human diseases, including cancer[140]. In the coming years, we expect to see intense research on the mechanisms by which malfunctions of chromatin regulators contribute to these diseases. Some chromatin alterations are potentially reversible, which raises the possibility of correcting chromatin states as a therapeutic strategy.

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