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Epigenetic regulation in pluripotent stem cells: a key to breaking the epigenetic barrier

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The differentiation and reprogramming of cells are accompanied by drastic changes in the epigenetic profiles of cells. Waddington's classical model clearly describes how differentiating cells acquire their cell identity as the developmental potential of an individual cell population declines towards the terminally differentiated state. The recent discovery of induced pluripotent stem cells as well as of somatic cell nuclear transfer provided evidence that the process of differentiation can be reversed. The identity of somatic cells is strictly protected by an epigenetic barrier, and these cells acquire pluripotency by breaking the epigenetic barrier by reprogramming factors such as Oct3/4, Sox2, Klf4, Myc and LIN28. This review covers the current understanding of the spatio-temporal regulation of epigenetics in pluripotent and differentiated cells, and discusses how cells determine their identity and overcome the epigenetic barrier during the reprogramming process.

1. Introduction

Induced pluripotent stem (iPS) cells are generated by the enforced expression of embryonic transcription factors, most commonly Oct3/4, Sox2, c-Myc and Klf4. In addition to pluripotency, they have infinite capacity for self-renewal [1,2]. iPS cells have been generated from multiple cell types, including keratinocytes [3], mesenchymal cells in fat [4], the oral mucosa [5], dental pulp cells [6], peripheral blood [7] and cord blood [8], as well as skin fibroblasts [2]. The characteristics of fully reprogrammed cells are functionally and molecularly very similar to those of embryonic stem (ES) cells in terms of their morphology, gene expression profile and capacity to differentiate into any of the three germ layers: endoderm, mesoderm and ectoderm. iPS cells could be a useful source for cell transplantation therapy, drug screening and disease modelling [9].

iPS cells are also highlighted as a cell model for epigenetic research. Pluripotent stem cells differentiate into any of the 200–300 specialized cell types with distinct properties. Waddington clearly described how differentiating cells acquire their cell identity, by illustrating differentiating cells as marbles rolling down valleys, with the developmental potential of individual cell populations declining towards the terminally differentiated state at the lowest elevation [10]. Recent genome-wide analyses using high-performance sequencers have uncovered key differences in the epigenetic landscape of pluripotent stem cells compared with that of lineage-committed cells. Waddington's classical model is now widely accepted; it appears that 'Waddington's marbles' are present in different valleys and that the different elevation levels have distinct epigenetic profiles, which are likely to play a role in the irreversibility of the properties of lineage-committed cells and the maintenance of their identity [11].

Cellular reprogramming induces differentiated cells to revert back to undifferentiated cells including pluripotent stem cells. On the basis of Waddington's model, somatic cells in differentiated states maintain their own cell fate and do not normally change from one differentiation pathway to another, although cell fate can be altered by nuclear reprogramming [11–15]. This reversal process can

be achieved by breaking the barrier of the differentiated state, and it provides one of the strategies for investigating the molecular basis of cell identity governed by epigenetic regulation. Because of its observed lower efficiency, the reprogramming process has been depicted as climbing a mountain, because it is much harder to achieve than differentiation, which is a spontaneous process, as sliding down a hill [11]. Therefore, a molecular understanding of the reprogramming process may address the question of how differentiated cells maintain their identity. Induced pluripotency is a process associated with gradual epigenetic changes [16], and thus can be exploited to obtain a molecular understanding of the determination of cell fate, which is mediated by epigenetic changes such as the silencing of retroviral transgenes upon the establishment of pluripotency [17,18], the reactivation of endogenous pluripotency genes [1], the establishment of bivalent chromatin domains in the promoters of developmentally regulated genes [12,19], global DNA hypomethylation, DNA hypermethylation of imprinted gene loci [17], reactivation of the inactive X chromosome in female iPS cells and reorganization of chromatin fibres [20,21].

This review summarizes studies performed to understand the epigenetic signatures associated with pluripotent and differentiated states, and addresses how their unique signatures contribute to the maintenance of pluripotency and how they are established during the reprogramming process.

2. Distinct histone modification profile in pluripotent cells

Recent technical advances have allowed us to map chromatin modifications throughout the genome by combining chromatin immunoprecipitation with DNA microarray analysis (ChIP-chip) or high-performance sequencing (ChIP-seq). Pluripotent stem cells have a unique expression pattern for histone modifiers and distinct distributions of modified histones.

The Polycomb group (PcG) complexes with the activity of H3K27 methylation to repress the expression of developmentally regulated genes in pluripotent stem cells [22,23], whereas the Trithorax group (TrxG) complexes with the activity of H3K4 methylation to activate the expression of genes associated with self-renewal [24]. An active mark, H3K4me3, is frequently observed in promoter regions of pluripotent stem cells, and is linked to transcriptional activation in general [25–27]. The methylation of H3K4 is mediated by TrxG members such as Set/mixed lineage leukaemia (MLL) methyltransferases. Wdr5, a key component of TrxG, interacts with H3K4me2, and mediates the transition of H3K4me2 to H3K4me3 [28]. The expression of Wdr5 is the highest in undifferentiated ES and iPS cells, and the level decreases during the differentiation process. The expression of Wdr5 along with the reprogramming factors enhances the efficiency of iPS cell generation [24]. Wdr5 physically interacts with Oct3/4, and co-occupies the DNA-binding sites of Oct3/4. Silencing of Wdr5 expression results in decreased expression of Oct3/4 target genes and the loss of self-renewal capacity of ES cells. H3K4 demethylase LSD1 stabilizes global DNA methylation [29] and also maintains an appropriate balance between H3K4 and H3K27 methylation in the regulatory regions of several developmental genes in pluripotent stem cells [30]. The recently reported interaction between LSD1 and Dnmt1 indicated that LSD1 mediates the linkage between DNA methylation and H3K4 demethylation [31].

The methylation of H3K27 is mediated by Polycomb repressive complex 2 (PRC2), which is composed of PcG proteins such as enhancer of zeste 2 (Ezh2), embryonic ectoderm development (Eed) and suppressor of zeste 12 homolog (Suz12) [32,33]. ES cells lacking a single component of the PRC2 complex, such as *Ezh2*, *Eed* or *Suz12*, show partial disruption of self-renewal accompanied by complete depletion of H3K27me3 [23,34], indicating that each component of the PRC2 complex collaboratively executes H3K27 trimethylation and regulates pluripotency and differentiation [35–38]. The histone methyltransferase activity of Ezh2 is responsible for maintaining H3K27 trimethylation in pluripotent stem cells [36,38,39]. Suz12 interacts with Ezh2, and inhibits protein degradation of Ezh2 [37]. A genome-wide analysis showed that Suz12 is co-localized with H3K27 trimethylation at key development regulators, as well as with highly conserved non-coding elements in ES cells [22]. A subset of Suz12-bound and H3K27me3-enriched genes are co-occupied by Oct3/4, Sox2 and Nanog. They are preferentially activated during ES cell differentiation, indicating that PRC2 poises differentiation-related genes for rapid gene activation during differentiation in pluripotent stem cells [22,40]. The PRC1 complexes composed of RING1A, RING1B, BMI1 and other proteins exhibit diverse functions in a PRC2-independent manner, such as ubiquitination of lysine 119 of H2A [41,42], and are also involved in the repression of transcription [43,44]. Previous studies in *Drosophila melanogaster* and *Caenorhabditis elegans* demonstrated that PcG proteins bind *cis*-acting DNA sequences and repress transcription, facilitating heterochromatin formation by binding to RNA [45–50]. For example, the incorporation of non-coding RNAs into PRC2 complexes has been observed. The PRC2 complexes interact with *Xist* RNA in mouse ES cells [51], whereas interaction between *HOTAIR* and SUZ12 has been observed in human fibroblasts. Such a gene repression mechanism may also be employed by mammalian pluripotent stem cells.

Transcriptionally inactive heterochromatin is usually accompanied by H3K9 di- and tri-methylation (H3K9me2/3). Oct3/4 upregulates demethylases for H3K9me2/3, such as *Jmjd1a* and *Jmjd2c*, by interacting with their promoters. Demethylation of H3K9me2/3 by these demethylases contributes to the self-renewal of ES cells [52,53]. In fact, depletion of *Jmjd1a* and *Jmjd2c* leads to decreased expression of pluripotency genes and differentiation of ES cells. In contrast, H3K9 methyltransferases have been reported to play an important role in early embryogenesis. G9a is an H3K9 methyltransferase that is essential for embryonic development [54], and has been shown to prevent reprogramming by recruiting Dnmt3a and Dnmt3b to the promoters of Oct3/4 and HP1β [55]. Treatment of cells with a chemical inhibitor specific for G9a increases the efficiency of iPS cell generation [56]. Although the molecular significance of silencing is unknown, ES cells are considered to be a good model for studying the relationship between DNA methylation and histone modifications, because of their high level of de novo DNA methyltransferase activity [57]. Endogenous retroviruses (ERVs) are transcriptionally silenced in ES cells. However, the silencing of ERVs is initiated by the H3K9 methyltransferase ESET/SETDB1, with KRAB-associated protein 1 (KAP1, also known as

TRIM28) in a DNA methylation-independent manner [58,59]. This suggests that not only the global level of H3K9me2/3, but also the context-dependent regulation of H3K9 (de)methylation is involved in the maintenance of pluripotency and differentiation. It is unclear whether the level of H3K9me2/3 is lower in pluripotent stem cells [60,61].

The acetylation of histones is also a significant modification observed in pluripotent stem cells. The level of acetylation is generally correlated with transcriptional activation, and is strictly regulated by the balanced actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) [62]. RNA interference screening of ES cells for chromatin components showed that a large set of HAT complexes to which Tip60 (TAT-interacting protein 60)/p400 contributes are ES cell development regulators, such as Gata4 and Gata6, and significantly overlap with target genes of Nanog [63,64]. On the other hand, HDAC inhibitors, such as valproic acid and trichostatin A, improve the efficiency of nuclear reprogramming by both nuclear transfer [65,66] and the transduction of pluripotency genes [67], suggesting that histone acetylation is involved in the maintenance and acquisition of pluripotency.

One of the most distinctive features of histone modifications in pluripotent stem cells is hypothesized to be 'bivalent domains', where both the active mark H3K4me3 and the repressive mark H3K27me3 are observed [25,68, 69]. These conflicting marks are preferentially observed at promoters of lineage-specific genes in pluripotent stem cells but very rarely in differentiated cells [19,25,27,68, 70,71]. This finding indicates that target genes in bivalent domains are 'poised' for expression, which is kept silent by H3K27 trimethylation in pluripotent stem cells and is presumably dependent on the trimethylation of H3K4. For example, while the expression of genes in bivalent domains is low in pluripotent stem cells, it switches to conventional patterns in the presence of active or repressive marks by erasing opposite marks during differentiation [69,72]. Consequently, differentiation-related genes with bivalent domains are expressed only in cells of their specific lineage. The repressive function of H3K27 methylation at lineage-specific loci is also demonstrated by the derepressed expression of these target genes in ES cells lacking key subunits of the H3K27 methyltransferase complex PRC2 [22,23,68]. Thus, the formation of poised chromatin architecture is proposed to be a key mechanism involved in both the maintenance of pluripotency and the developmental potential of pluripotent stem cells.

Incomplete formation of bivalent domains is occasionally observed in partially reprogrammed cells [19]. Furthermore, the *Ink4*/*Arf* locus is silenced during the early stage of reprogramming with the formation of bivalent chromatin domains, whereas forced silencing of *Ink4*/*Arf* by shRNA increases the efficiency of iPS cell generation, indicating that the *Ink4*/*Arf* locus could functionally behave as a barrier to reprogramming [73]. In this way, genes responsible for differentiation are susceptible to the formation of bivalent domains, and keep target genes poised for transcriptional activation in pluripotent stem cells [12].

In the last decade, it has come to be widely accepted that the bivalent domain is one of the most distinctive features of pluripotent stem cells. However, a recent study revised the role of the bivalent domain in pluripotent stem cells. Mouse ES cells cultured in leukaemia inhibitory factor (LIF)-containing and feeder-free medium with two small-molecule kinase inhibitors (2i) exhibited ground-state pluripotency. These naive ES cells exhibited a decreased amount of H3K27me3 on the bivalent domain compared with that observed in mouse ES cells under conventional culture conditions. The distribution of H3K4me3 in naive ES cells is similar to that in ES cells under 2i-free culture conditions, demonstrating that the bivalent domain is transiently formed during differentiation [74].

The replacement of canonical histones with specific variant forms has emerged as a key mechanism of modulation of nucleosome dynamics and chromatin structure. Incorporation of histone variants alters the interaction surfaces and overall stability of nucleosomes, including localized changes in chromatin structure and the formation of specialized chromosomal domains [75–77]. Some of the histone variants are considered to play an important role in differentiation or reprogramming. In ES cells, H2AZ, a highly conserved variant of H2A, is preferentially incorporated into the bivalent domains of developmentally important genes [78,79]. The depletion of H2AZ by RNA interference in ES cells results in the expulsion of PcG proteins from the bivalent domains, leading to derepression of genes that are silenced by the PcG complexes, although H2AZ is not required for the maintenance of stemness in ES cells [78].

In addition to H2AZ, macroH2A, one of the histone variants incorporated mainly in heterochromatin [80], was recently identified as a regulator of reprogramming [81]. In addition to variants of core histones, differential composition of a linker histone H1 has been observed [82]. The possible roles of other histone variants in differentiation or reprogramming also need to be evaluated.

3. DNA methylation and demethylation: modulating the barrier for reprogramming

DNA methylation maintains long-lasting cell memories, and is therefore considered to be a pivotal epigenetic barrier to cellular reprogramming [83]. During reprogramming, the activation of endogenous pluripotency genes including *Oct3/4* and *Nanog* is accompanied by erasing the methylation of cytosines at their promoter regions. Insufficient DNA demethylation at the promoter regions, which is occasionally observed in partially reprogrammed iPS cells, fails to produce the robust reactivation of pluripotency genes [1,84–86]. In addition, the differential patterns of DNA methylation that are associated with genomic imprinting, retrotransposon silencing and X chromosome inactivation are observed between differentiated and pluripotent stem cells and among a series of pluripotent stem-cell lines [27,86–89], indicating that DNA methylation may be a suitable epigenetic marker for characterizing pluripotent stem-cell lines. Although it is unclear how such differential levels of DNA methylation arise, functional linkage between DNA methylation and reprogramming has been demonstrated. The inhibition of DNA methylation by chemical compounds or RNA interference targeting DNA methyltransferase can increase the efficiency of iPS cell generation [19].

Recent analyses using a high-performance sequencer have enabled mapping of DNA methylation with high resolution and have revealed an intriguing distribution of methylated cytosine in pluripotent stem cells. Since DNA methylation

is frequently observed at CpG islands, which contain a high frequency of CpG sites, it is considered that the frequency of CpG sequences was positively correlated with the susceptibility to DNA methylation. However, the most recent studies of genome-wide DNA methylation status in pluripotent stem cells have produced observations that differ from the widely accepted model. The methylation levels of CpGs in pluripotent stem cells were negatively correlated with the local CpG density.

In ES and iPS cells, regions with high CpG density exhibited low DNA methylation, whereas those with low CpG density exhibited high DNA methylation [27,87,90,91]. Regions with low CpG density are frequently observed in the promoters of tissue-specific genes [91], implying that the mechanism responsible for DNA methylation in the regulation of tissue-specific genes is different from that for DNA methylation in the regulation of other genes. Intriguingly, DNA hypermethylation at the promoters of these tissue-specific genes with low CpG density is accompanied by bivalent chromatins in ES and iPS cells [91,92]. The relevance of this uniquely low CpG methylation level in pluripotent stem cells with bivalent domains is yet to be investigated at the molecular level; such information would provide important clues regarding the mechanisms of epigenetic regulation during differentiation.

A single-base-resolution methylome analysis by whole-genome bisulphite sequencing (WGBS) also highlighted the significance of non-CG methylation in pluripotent stem cells [86,89]. Surprisingly, approximately one-quarter of all methylated cytosines in ES and iPS cells occurred in a non-CpG context, whereas most of the methylated cytosines in somatic cells were observed in CpG sequences. These pluripotent stem cell-specific non-CpG methylation sites tend to be located in the exonic regions of actively transcribed genes [86]. The existence of DNA methylation in cytosine of non-CpG may be linked to the fidelity of DNA methylation, which was proposed in a previous study of DNA demethylase Tet1 [93].

Studies using mice harbouring mutant DNA methyltransferases showed the importance of strict regulation of DNA methylation during the normal developmental process. Dnmt1 and Dnmt3a/Dnmt3b are enzymes essential for the maintenance and establishment of DNA methylation, respectively [87,94,95]. The loss of *Dnmt1* causes the loss of two-thirds of total DNA methylation, thus leading to embryonic lethality [96]. Embryos with mutant *Dnmt3b* appear to be normal in early developmental stages but show multiple developmental defects in the later stages [97]. The conditional deletion of *Dnmt3b* in mouse embryonic fibroblasts leads to a partial loss of DNA methylation [98]. However, although the Dnmt family plays an essential role in both the developmental process and the reprogramming of germ cells, de novo methylation by Dnmt3a and Dnmt3b is dispensable for the induction of iPS cells [99].

The mechanism by which methylated cytosine is converted into unmodified cytosine during the reprogramming process is elusive. However, with regard to DNA demethylation at the global level, two possible mechanisms have been proposed: a replication-independent 'active' DNA demethylation pathway and a replication-dependent 'passive' DNA demethylation pathway. The existence of active DNA demethylation is demonstrated by the base-excision repair (BER) machinery in plants and in fertilized eggs of animals. Previous studies have suggested TDG [100,101], MBD4 [102], AID/APOBEC [103], GADD45A [104] and MBD2B [105–107] as candidate DNA demethylases in mammalian cells [108–111], and the coordinated action of these factors is required for active DNA demethylation through the BER machinery [112]. However, the roles of these molecules in active DNA demethylation in mammals are controversial.

The recent findings of Tet family proteins as candidate DNA demethylases have advanced our understanding of DNA demethylation in pluripotent stem cells and other tissues [113–119]. Tet family proteins catalyze the conversion of methylcytosine to 5-hydroxymethylcytosine (5hmC) in an Fe(II)- and α-ketoglutarate-dependent manner. Tet proteins have been implicated in ES cell maintenance and lineage specification *in vitro*. Tet1 and Tet2 are highly expressed in mouse ES cells, and are downregulated upon cell differentiation [116,119]. The silencing of *Tet1* expression by RNA interference downregulates the expression of pluripotency genes such as *Nanog*, *Esrrb*, *Klf4*, *Prdm14*, *Lefty1* and *Lefty2*, and increases the trans-differentiation potential of ES cells to extra-embryonic lineages [93,116,119–121]. Genome-wide analysis using a high-performance sequencer revealed the presence of an intricate relationship between Tet1 and the expression of its target genes [93,120,122,123].

Tet1 preferentially binds to the gene body and GC-rich sequences in promoter regions of both transcriptionally active and repressive genes [122]. The Tet1-binding sites overlap with PcG-target sites [123]. Consistently, the proteomic analyses identified Sin3a, a component of the PcG protein complex, as a binding partner of Tet1 [93]. Knockdown of Tet1 decreases the expression of PcG-target genes and pluripotency-related genes [120], indicating that gene regulation by Tet1 cannot be completely explained by the collaborative functioning with PcG. The involvement of chromatin remodellers in Tet1-mediated gene regulation has also been reported. The Mbd3/NURD complexes were identified as a 'reader' of 5hmC. The Mbd3/NURD complexes directly recognize 5hmC and control the expression of Tet1-target genes [124]. Functioning of Mbd3/NURD complexes as a reader of 5hmC may affect the regulation of Tet1-target genes through modification with 5hmC. Knockdown of Tet1 in ES cells produces a phenotype similar to that of Mbd3-knockdown ES cells with increased expression of trophectoderm markers, implying a functional link between Tet1 and Mbd3. In addition to 5hmC, 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) were detected as intermediates of the oxidation reaction mediated by Tet1 [125], and 5caC is subject to base excision by thymine–DNA glycosylase (TDG) in ES cells [126]. This suggests that the oxidation of Tet1 followed by base excision of 5caC by TDG is a possible pathway for active DNA demethylation in ES cells. The functional relevance of Tet1 in active DNA demethylation and the maintenance of pluripotency has been demonstrated *in vitro*; however, Tet1 mutant mice are viable and fertile. Moreover, ES cells from mutant mice did not show any aberrations in the maintenance of pluripotency [127]. Although the involvement of Tet1-mediated active DNA demethylation in the maintenance of pluripotency is of great interest, further studies are necessary to elucidate the role(s) of Tet1 in pluripotent stem cells.

4. Implication of other epigenetic regulations: chromatin remodelling, high-order structure and non-coding RNA

Adenosine triphosphate (ATP)-dependent chromatin remodelling factors, which are capable of mobilizing or displacing nucleosomes at both the global and the locus-specific level [128–130], regulate gene expression programmes in early development and cell fate decisions [129,131,132].

SWI/SNF (switch/sucrose non-fermentable) complexes trigger the ejection of nucleosomes. The SWI/SNF factor is composed of two complexes Brg/Brahma-associated factors (BAF) and polybromo BAF (PBAF), and contributes to the self-renewal, proliferation or differentiation of ES cells. BRG1, a catalytic subunit of BAF, regulates the self-renewal and pluripotency of ES cells [133]. Knockdown of BRG1 by RNA interference results in morphological changes and a decreased proliferation of ES cells. BRG1 binds to the promoter regions of pluripotency genes such as *Oct3/4*, *Sox2* and *Nanog*. Decreased expression of BRG1 downregulates the expression of pluripotency genes including *Oct3/4*, *Sox2* and *Sall4*, accompanied by upregulation of differentiation-related genes such as *Gata4* and *Gata6*. The involvement of esBAF, an ES cell-specific BAF complex, in self-renewal and pluripotency has also been reported [134–137]. esBAF enhances the binding of Oct3/4 to the target promoters, and facilitates the reprogramming of fibroblasts [138]. Although the molecular mechanism of esBAF-mediated facilitation of reprogramming has been explained, Brg1, the subunit of esBAF, also facilitates PcG function and represses the expression of classical PcG targets such as Hox genes, the expression of which is essential for the maintenance of pluripotency [139]. The CHD (chromodomain helicase DNA-binding) family complexes, as well as SWI/SNF complexes, trigger the ejection of nucleosomes, and are involved in the cell identity and function of ES cells [140–146]. CHD complexes are composed of the CHD enzymes, methyl-CpG-binding domain 3 (MBD3) and HDACs. The NuRD complex includes CHD3, CHD4 and MBD3, and is responsible for the deacetylation and trimethylation of H3K27. In addition, the NuRD complex is essential for maintaining both pluripotency and developmental transitions in early embryogenesis [140,147,148]. In addition to the function of the complexes, the roles of each component of the complexes in pluripotency have been reported. For example, CHD1 targets Oct3/4-binding sites, and is required for efficient reprogramming of fibroblasts to the pluripotent stem-cell state. The silencing of CHD1 expression by RNA interference blocks normal differentiation and the accumulation of heterochromatin [144]. The deletion of HDAC1 results in aberrant differentiation of ES cells, the effects of which include preferential differentiation toward the mesodermal and ectodermal lineages at the expense of endoderm [149], and leads to embryonic lethality [150–153]. ES cells lacking MBD3 express trophectodermal markers and show aberrant differentiation with sustained high expression of Oct3/4 [140,146]. The restriction of interaction of MBD3 with the SWI/SNF component BRG1 to pluripotent stem cells [137] implies that crosstalk among chromatin remodelling complexes regulates the pluripotency of the cells.

The TIP60/p400 complexes belonging to the INO80 family regulate gene transcription by depositioning histone variants H2A.Z into chromatin [130]. Knockdown of Tip60/p400 expression in ES cells resulted in aberrant morphology and a loss of pluripotency. The expression profile of Tip60/p400-silenced cells was similar to that of *Nanog*-silenced cells [63], suggesting that Nanog and Tip60/p400 cooperatively maintain the pluripotency of ES cells. Bprt, a member of the ISWI family proteins, is also involved in early embryonic growth and represses the expression of differentiation markers in ES cells [154].

The organization of high-order chromatin structures has emerged as a key machinery of genome regulation [155–157]. ES cells possess loosely compacted euchromatin. They have an increased level of highly condensed heterochromatin that forms transcriptionally inactive regions during the differentiation process [158,159]. Another study using fully reprogrammed iPS cells with high Nanog expression and partially reprogrammed iPS cells that were morphologically similar to ES cells but lacked Nanog expression revealed that fully reprogrammed cells with high pluripotency lose the ability to form heterochromatin [21]. Not only loci-specific heterochromatin formation, but also other nuclear features, such as the nuclear lamina nucleolus and nuclear speckles, may affect the chromatin architecture. However, the role of the lamina in pluripotency remains controversial. One report showed morphological differences between pluripotent and differentiated cells [20], whereas another report indicated that B-type laminas are not required for ES cells [160].

RNA occasionally acts as a chromatin regulator. MicroRNAs (miRNAs) regulate the post-transcriptional control of gene expression [161]. The involvement of miRNAs in the maintenance of pluripotency was suggested by the finding that the expression of miRNAs is regulated by the core transcriptional regulatory circuit in ES cells [162]. Studies using mice lacking either Dicer or Dgcr8, which are required for the maturation of all miRNAs, reveal that they play essential roles in the proliferation and differentiation of ES cells [163–165]. However, although the loss of Dgcr8 results in a cell cycle defect and aberrant differentiation as a result of inability to silence the self-renewal programme of ES cells, *Dgcr8*-deficient mice still maintain self-renewal in ES cells [163]. This complicated regulation of pluripotency by miRNA is now partially explained by antagonism between miRNA-294, a regulator of the cell cycle in ES cells, and the *let-7* family, which is abundantly expressed in somatic cells, in the stabilization of the self-renewing and differentiation status [166]. miRNA-294 downregulates the *let-7* family through stabilization of the *let-7*-negative regulator LIN28 in the self-renewal state. LIN28 is highly expressed in pluripotent stem cells [167,168] and facilitates the reprogramming of somatic cells in collaboration with other pluripotency genes, such as Oct3/4, Sox2 and Nanog [169]. *let-7* downregulates MYC in cancer cells through let7-binding sites on MYC 3′UTR, whereas the overexpression of MYC decreases let-7 expression [170,171]. These observations reveal a direct double-negative feedback loop, and imply similar capacities of MYC and LIN28 to promote induction of pluripotency. However, further study is required, particularly to characterize the autoregulatory loop between MYC and LIN28 in pluripotent stem cells.

Although previous studies have demonstrated similar expression profiles of mRNA and miRNA in different clones of both ES cells and iPS cells, other studies have shown differential expression of a few transcripts from imprinted regions or pluripotency-related genes. Some iPS

cell clones display aberrant silencing at the *Dlk-Dio3* gene cluster on mouse chromosome 12F1, which is associated with a poor cellular contribution to chimeric mice [172]. However, another report demonstrated contradictory evidence in which the stoichiometry of reprogramming factors, but not the imprinting status at the *Dlk1-Dio3* region, strongly interfered with the quality of iPS cells [160]. Hence, further studies using a number of iPS cells with well-characterized methodology are required to elucidate the molecular features that can predict the quality of pluripotent stem cells.

X chromosome inactivation (XCI) is a regulatory mechanism by which one of the two X chromosomes in female cells is silenced [173,174]. A mouse ES cell or fully reprogrammed iPS cell carries two active chromosomes (XaXa) and the differentiation of these cells initiates XCI, leading to the inactivation of either X chromosome to equalize the expression of X-linked genes between the male (XY) and the female (XX). The reprogramming of female mouse fibroblasts faithfully reactivated the silenced X chromosome [175]. However, a previous study showed that some human ES cells and iPS cells from female cells retained an inactive X chromosome [176]. Human pluripotent stem cells share characteristics with mouse epiblast stem cells (EpiSCs), suggesting that human pluripotent stem cells are in a 'primed' pluripotent state, whereas mouse ES cells and iPS cells are in a 'naive' pluripotent state [177]. Since diverse mechanisms for the initiation of XCI during development have been found in mammals, such differences may be associated with the observed inconsistencies in X chromosome reactivation between mouse and human pluripotent stem cells [178]. There are recent reports of human iPS cells with ground-state pluripotency, demonstrated by X chromosome reactivation, under certain culture conditions [179,180]. Further studies are required for understanding of the molecular mechanism responsible for the inactivation and reactivation of the X chromosome during the reprogramming process [181].

5. Conclusions and perspectives

Pluripotent stem cells have been used as a cell model for understanding the molecular mechanism of cellular differentiation and a source of cells for regenerative medicine. Studies of stem-cell identity and the fate of pluripotent stem cells upon differentiation have advanced remarkably over the last few decades. Many studies, including a recent genome-wide analysis of epigenetic modifications, support the classical 'landscape model' of Waddington, which describes irreversible cell differentiation. Our growing understanding of epigenetic regulation in pluripotent stem cells and their dynamic changes during differentiation can be used to update this model, which represents not only cell fate but also the coupling of developmental potential with the epigenetic status of the cells during differentiation.

The recent discovery of iPS cells has enabled us to dissect epigenetic regulation during reprogramming and differentiation [1,182]. Reprogramming, the reverse of differentiation, is achieved by breaking the barrier of the differentiated state. Dissection of epigenetic regulation during the reprogramming process may provide a description of how cells sustain their fate and may provide candidates for molecules that act as guardians of differentiation. The identification of guardian molecules that are responsible for the differentiated state of cells will be of use in the efficient generation of iPS cells. Reprogramming factors, including Oct3/4 and Sox2, are thought to be inducers of pluripotency and may also act as 'destroyers' of the differentiated state. It will be of interest to know how reprogramming factors contribute to the destruction of the epigenetic barrier in the early stage of the reprogramming process, and whether such a mechanism directly regulates the master regulators that maintain the differentiated state, or break the differentiated state through genome-wide alteration of epigenetic status.

Pluripotent stem cells are now suggested as an artificial source of tissues, and consequently it is necessary to be able to guarantee their safety in the human body after transplantation. However, both ES cells and iPS cells are produced after long-term culture, and thus harbour clone-to-clone variations in their epigenetic profiles as well as DNA sequences and copy numbers. Difference in iPS cells among clones have been reported [85], and it is therefore important to validate the quality of pluripotent stem cells including ES and iPS cells by genomic and epigenomic analyses.

Methylome analysis may be a good candidate for evaluating the quality of pluripotent stem cells, since DNA methylation is stable and acts as a source of long-term memory. Some residual DNA methylation signatures observed in iPS cells show characteristics of their somatic tissues of origin, implying the presence of epigenetic memory [85,183]. Considering the recent reports of variation among clones in terms of the characteristics of pluripotent stem cells, it is crucial to establish methods for the reliable evaluation of the quality of iPS cells, which should eventually be useful for generating clinical-grade iPS cells for use in regenerative medicine.

Analysis using deep sequencers has also revealed non-negligible differences among individuals in the genome and epigenome. One of the advantages of using iPS cells as pluripotent stem cells is the ability to analyse and compare established iPS cells with the original somatic cells. In other words, with these cells, it is possible to distinguish whether the observed alterations in the genome/epigenome represent aberrations acquired during long-term cell culture or the individual variation that is normally observed among the individuals.

There is increasing evidence of the importance of epigenetic regulation in maintaining pluripotency and the reprogramming process. Current high-performance sequencers make it possible to screen for genomic alterations at the whole-genome level, and can be used to guarantee that the cells are of clinical grade, on the basis of their genomic sequence. It is also important to examine the epigenetic profile of pluripotent stem cells, because the epigenetic landscape represents both the past and the current developmental state, and may be a useful indicator to predict their future potential. Further advances in the understanding of epigenetic regulation hold promise for the molecular understanding of cell fate and the realization of regenerative medicine using pluripotent stem cells.

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References

1. Takahashi K, Yamanaka S. 2006 Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676. (doi:10.1016/j.cell.2006.07.024)
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. 2007 Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872. (doi:10.1016/j.cell.2007.11.019)
3. Aasen T *et al*. 2008 Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat. Biotechnol.* **26**, 1276–1284. (doi:10.1038/nbt.1503)
4. Sun NF *et al*. 2009 Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. *Proc. Natl Acad. Sci. USA* **106**, 15 720–15 725. (doi:10.1073/pnas.0908450106)
5. Miyoshi K, Tsuji D, Kudoh K, Satomura K, Muto T, Itoh K, Noma T. 2010 Generation of human induced pluripotent stem cells from oral mucosa. *J. Biosci. Bioeng.* **110**, 345–350. (doi:10.1016/j.jbiosc.2010.03.004)
6. Tamaoki N *et al*. 2010 Dental pulp cells for induced pluripotent stem cell banking. *J. Dental Res.* **89**, 773–778. (doi:10.1177/0022034510366846)
7. Loh YH *et al*. 2009 Generation of induced pluripotent stem cells from human blood. *Blood* **113**, 5476–5479. (doi:10.1182/blood-2009-02-204800)
8. Giorgetti A *et al*. 2009 Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. *Cell Stem Cell* **5**, 353–357. (doi:10.1016/j.stem.2009.09.008)
9. Nishikawa S, Goldstein RA, Nierras CR. 2008 The promise of human induced pluripotent stem cells for research and therapy. *Nat. Rev. Mol. Cell Biol.* **9**, 725–729. (doi:10.1038/nrm2466)
10. Waddington CH. 1957 *The strategy of the genes*. London: George Allen & Unwin.
11. Hochedlinger K, Plath K. 2009 Epigenetic reprogramming and induced pluripotency. *Development* **136**, 509–523. (doi:10.1242/dev.020867)
12. Spivakov M, Fisher AG. 2007 Epigenetic signatures of stem-cell identity. *Nat. Rev. Genet.* **8**, 263–271. (doi:10.1038/nrg2046)
13. Lunyak VV, Rosenfeld MG. 2008 Epigenetic regulation of stem cell fate. *Hum. Mol. Genet.* **17**, R28–R36. (doi:10.1093/hmg/ddn149)
14. Meissner A. 2010 Epigenetic modifications in pluripotent and differentiated cells. *Nat. Biotechnol.* **28**, 1079–1088. (doi:10.1038/nbt.1684)
15. Pasque V, Jullien J, Miyamoto K, Halley-Stott RP, Gurdon JB. 2011 Epigenetic factors influencing resistance to nuclear reprogramming. *Trends Genet.* **27**, 516–525. (doi:10.1016/j.tig.2011.08.002)
16. Djuric U, Ellis J. 2010 Epigenetics of induced pluripotency, the seven-headed dragon. *Stem Cell Res. Therapy* **1**, 3. (doi:10.1186/scrt3)
17. Okita K, Ichisaka T, Yamanaka S. 2007 Generation of germline-competent induced pluripotent stem cells. *Nature* **448**, 313–317. (doi:10.1038/nature05934)
18. Jaenisch R, Young R. 2008 Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* **132**, 567–582. (doi:10.1016/j.cell.2008.01.015)
19. Mikkelsen TS *et al*. 2008 Dissecting direct reprogramming through integrative genomic analysis. *Nature* **454**, 49–55. (doi:10.1038/nature07056)
20. Meshorer E, Misteli T. 2006 Chromatin in pluripotent embryonic stem cells and differentiation. *Nat. Rev. Mol. Cell Biol.* **7**, 540–546. (doi:10.1038/nrm1938)
21. Fussner E, Djuric U, Strauss M, Hotta A, Perez-Iratxeta C, Lanner F, Dilworth FJ, Ellis J, Bazett-Jones DP. 2011 Constitutive heterochromatin reorganization during somatic cell reprogramming. *EMBO J.* **30**, 1778–1789. (doi:10.1038/emboj.2011.96)
22. Lee TI *et al*. 2006 Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* **125**, 301–313. (doi:10.1016/j.cell.2006.02.043)
23. Boyer LA *et al*. 2006 Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* **441**, 349–353. (doi:10.1038/nature04733)
24. Ang YS *et al*. 2011 Wdr5 mediates self-renewal and reprogramming via the embryonic stem cell core transcriptional network. *Cell* **145**, 183–197. (doi:10.1016/j.cell.2011.03.003)
25. Bernstein BE *et al*. 2006 A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315–326. (doi:10.1016/j.cell.2006.02.041)
26. Mikkelsen TS *et al*. 2007 Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553–560. (doi:10.1038/nature06008)
27. Meissner A *et al*. 2008 Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* **454**, 766–770. (doi:10.1038/nature07107)
28. Wysocka J, Swigut T, Milne TA, Dou Y, Zhang X, Burlingame AL, Roeder RG, Brivanlou AH, Allis CD. 2005 WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. *Cell* **121**, 859–872. (doi:10.1016/j.cell.2005.03.036)
29. Wang J *et al*. 2009 The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat. Genet.* **41**, 125–129. (doi:10.1038/ng.268)
30. Adamo A, Sese B, Boue S, Castano J, Paramonov I, Barrero MJ, Belmonte JCI. 2011 LSD1 regulates the balance between self-renewal and differentiation in human embryonic stem cells. *Nat. Cell Biol.* **13**, 652–659. (doi:10.1038/ncb2246)
31. Clements EG, Mohammad HP, Leadem BR, Easwaran H, Cai Y, Van Neste L, Baylin SB. 2012 DNMT1 modulates gene expression without its catalytic activity partially through its interactions with histone-modifying enzymes. *Nucleic Acids Res.* **40**, 4334–4346. (doi:10.1093/nar/gks031)
32. Cao R, Zhang Y. 2004 SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED–EZH2 complex. *Mol. Cell* **15**, 57–67. (doi:10.1016/j.molcel.2004.06.020)
33. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. 2002 Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**, 1039–1043. (doi:10.1126/science.1076997)
34. Chamberlain SJ, Yee D, Magnuson T. 2008 Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. *Stem Cells* **26**, 1496–1505. (doi:10.1634/stemcells.2008-0102)
35. Faust C, Schumacher A, Holdener B, Magnuson T. 1995 The eed mutation disrupts anterior mesoderm production in mice. *Development* **121**, 273–285.
36. O'Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, Jenuwein T. 2001 The polycomb-group gene Ezh2 is required for early mouse development. *Mol. Cell. Biol.* **21**, 4330–4336. (doi:10.1128/MCB.21.13.4330-4336.2001)
37. Pasini D, Bracken AP, Jensen MR, Lazzerini Denchi E, Helin K. 2004 Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J.* **23**, 4061–4071. (doi:10.1038/sj.emboj.7600402)
38. Shen X, Liu Y, Hsu YJ, Fujiwara Y, Kim J, Mao X, Yuan G-C, Orkin SH. 2008 EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol. Cell* **32**, 491–502. (doi:10.1016/j.molcel.2008.10.016)
39. Erhardt S *et al*. 2003 Consequences of the depletion of zygotic and embryonic enhancer of zeste 2 during preimplantation mouse development. *Development* **130**, 4235–4248. (doi:10.1242/dev.00625)
40. Boyer LA *et al*. 2005 Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947–956. (doi:10.1016/j.cell.2005.08.020)
41. Bracken AP, Dietrich N, Pasini D, Hansen KH, Helin K. 2006 Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. *Genes Dev.* **20**, 1123–1136. (doi:10.1101/gad.381706)
42. Schoeftner S, Sengupta AK, Kubicek S, Mechtler K, Spahn L, Koseki H, Jenuwein T, Wutz A. 2006 Recruitment of PRC1 function at the initiation of X inactivation independent of PRC2 and silencing. *EMBO J.* **25**, 3110–3122. (doi:10.1038/sj.emboj.7601187)
43. Cao R, Tsukada Y, Zhang Y. 2005 Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene

silencing. *Mol. Cell* **20**, 845–854. (doi:10.1016/j.molcel.2005.12.002)

44. Jorgensen HF, Giadrossi S, Casanova M, Endoh M, Koseki H, Brockdorff N, Fisher AG. 2006 Stem cells primed for action: polycomb repressive complexes restrain the expression of lineage-specific regulators in embryonic stem cells. *Cell Cycle* **5**, 1411–1414. (doi:10.4161/cc.5.13.2927)
45. Min J, Zhang Y, Xu RM. 2003 Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. *Genes Dev.* **17**, 1823–1828. (doi:10.1101/gad.269603)
46. Dellino GI, Schwartz YB, Farkas G, McCabe D, Elgin SC, Pirrotta V. 2004 Polycomb silencing blocks transcription initiation. *Mol. Cell* **13**, 887–893. (doi:10.1016/S1097-2765(04)00128-5)
47. Wang L, Brown JL, Cao R, Zhang Y, Kassis JA, Jones RS. 2004 Hierarchical recruitment of polycomb group silencing complexes. *Mol. Cell* **14**, 637–646. (doi:10.1016/j.molcel.2004.05.009)
48. Mohd-Sarip A, van der Knaap JA, Wyman C, Kanaar R, Schedl P, Verrijzer CP. 2006 Architecture of a polycomb nucleoprotein complex. *Mol. Cell* **24**, 91–100. (doi:10.1016/j.molcel.2006.08.007)
49. Zhang H, Christoforou A, Aravind L, Emmons SW, van den Heuvel S, Haber DA. 2004 The *C. elegans* Polycomb gene SOP-2 encodes an RNA binding protein. *Mol. Cell* **14**, 841–847. (doi:10.1016/j.molcel.2004.06.001)
50. Hekimoglu B, Ringrose L. 2009 Non-coding RNAs in polycomb/trithorax regulation. *RNA Biol.* **6**, 129–137. (doi:10.4161/rna.6.2.8178)
51. Zhao J, Sun BK, Erwin JA, Song JJ, Lee JT. 2008 Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. *Science* **322**, 750–756. (doi:10.1126/science.1163045)
52. Loh YH, Zhang W, Chen X, George J, Ng HH. 2007 Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. *Genes Dev.* **21**, 2545–2557. (doi:10.1101/gad.1588207)
53. Ma DK, Chiang CH, Ponnusamy K, Ming GL, Song H. 2008 G9a and Jhdm2a regulate embryonic stem cell fusion-induced reprogramming of adult neural stem cells. *Stem Cells* **26**, 2131–2141. (doi:10.1634/stemcells.2008-0388)
54. Tachibana M *et al*. 2002 G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev.* **16**, 1779–1791. (doi:10.1101/gad.989402)
55. Epsztejn-Litman S *et al*. 2008 De novo DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. *Nat. Struct. Mol. Biol.* **15**, 1176–1183. (doi:10.1038/nsmb.1476)
56. Vedadi M *et al*. 2011 A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. *Nat. Chem. Biol.* **7**, 566–574. (doi:10.1038/nchembio.599)
57. Maksakova IA, Mager DL, Reiss D. 2008 Keeping active endogenous retroviral-like elements in check: the epigenetic perspective. *Cell. Mol. Life Sci.* **65**, 3329–3347. (doi:10.1007/s00018-008-8494-3)
58. Rowe HM *et al*. 2010 KAP1 controls endogenous retroviruses in embryonic stem cells. *Nature* **463**, 237–240. (doi:10.1038/nature08674)
59. Matsui T *et al*. 2010 Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. *Nature* **464**, 927–931. (doi:10.1038/nature08858)
60. Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP. 2009 Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat. Genet.* **41**, 246–250. (doi:10.1038/ng.297)
61. Filion GJ, van Steensel B. 2010 Reassessing the abundance of H3K9me2 chromatin domains in embryonic stem cells. *Nat. Genet.* **42**, 4 author reply 5–6. (doi:10.1038/ng0110-4)
62. Yang XJ, Seto E. 2008 Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol. Cell* **31**, 449–461. (doi:10.1016/j.molcel.2008.07.002)
63. Fazzio TG, Huff JT, Panning B. 2008 An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity. *Cell* **134**, 162–174. (doi:10.1016/j.cell.2008.05.031)
64. Chen X *et al*. 2008 Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell* **133**, 1106–1117. (doi:10.1016/j.cell.2008.04.043)
65. Kishigami S, Mizutani E, Ohta H, Hikichi T, Thuan NV, Wakayama S, Bui H-T, Wakayama T. 2006 Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer. *Biochem. Biophys. Res. Commun.* **340**, 183–189. (doi:10.1016/j.bbrc.2005.11.164)
66. Bui HT, Wakayama S, Kishigami S, Park KK, Kim JH, Thuan NV, Wakayama T. 2010 Effect of trichostatin A on chromatin remodeling, histone modifications, DNA replication, and transcriptional activity in cloned mouse embryos. *Biol. Reprod.* **83**, 454–463. (doi:10.1095/biolreprod.109.083337)
67. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. 2008 Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat. Biotechnol.* **26**, 795–797. (doi:10.1038/nbt1418)
68. Azuara V *et al*. 2006 Chromatin signatures of pluripotent cell lines. *Nat. Cell Biol.* **8**, 532–538. (doi:10.1038/ncb1403)
69. Pan G, Tian S, Nie J, Yang C, Ruotti V, Wei H, Jonsdottir GA, Stewart R, Thomson JA. 2007 Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell Stem Cell* **1**, 299–312. (doi:10.1016/j.stem.2007.08.003)
70. Chambeyron S, Da Silva NR, Lawson KA, Bickmore WA. 2005 Nuclear re-organisation of the Hoxb complex during mouse embryonic development. *Development* **132**, 2215–2223. (doi:10.1242/dev.01813)
71. Szutorisz H, Canzonetta C, Georgiou A, Chow CM, Tora L, Dillon N. 2005 Formation of an active tissue-specific chromatin domain initiated by epigenetic marking at the embryonic stem cell stage. *Mol. Cell. Biol.* **25**, 1804–1820. (doi:10.1128/MCB.25.5.1804-1820.2005)
72. Pasini D *et al*. 2010 JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature* **464**, 306–310. (doi:10.1038/nature08788)
73. Li H, Collado M, Villasante A, Strati K, Ortega S, Canamero M, Blasco MA, Serrano M. 2009 The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature* **460**, 1136–1139. (doi:10.1038/nature08290)
74. Marks H *et al*. 2012 The transcriptional and epigenomic foundations of ground state pluripotency. *Cell* **149**, 590–604. (doi:10.1016/j.cell.2012.03.026)
75. Henikoff S, Furuyama T, Ahmad K. 2004 Histone variants, nucleosome assembly and epigenetic inheritance. *Trends Genet.* **20**, 320–326. (doi:10.1016/j.tig.2004.05.004)
76. Jin J, Cai Y, Li B, Conaway RC, Workman JL, Conaway JW, Kusch T. 2005 In and out: histone variant exchange in chromatin. *Trends Biochem. Sci.* **30**, 680–687. (doi:10.1016/j.tibs.2005.10.003)
77. Boulard M, Bouvet P, Kundu TK, Dimitrov S. 2007 Histone variant nucleosomes: structure, function and implication in disease. *Sub-cellular Biochem.* **41**, 71–89.
78. Creyghton MP, Markoulaki S, Levine SS, Hanna J, Lodato MA, Sha K, Young RA, Jaenisch R, Boyer LA. 2008 H2AZ is enriched at polycomb complex target genes in ES cells and is necessary for lineage commitment. *Cell* **135**, 649–661. (doi:10.1016/j.cell.2008.09.056)
79. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. 2007 High-resolution profiling of histone methylations in the human genome. *Cell* **129**, 823–837. (doi:10.1016/j.cell.2007.05.009)
80. Banaszynski LA, Allis CD, Lewis PW. 2010 Histone variants in metazoan development. *Dev. Cell* **19**, 662–674. (doi:10.1016/j.devcel.2010.10.014)
81. Pasque V, Gillich A, Garrett N, Gurdon JB. 2011 Histone variant macroH2A confers resistance to nuclear reprogramming. *EMBO J.* **30**, 2373–2387. (doi:10.1038/emboj.2011.144)
82. Terme JM, Sese B, Millan-Arino L, Mayor R, Belmonte JC, Barrero MJ, Jordan A. 2011 Histone H1 variants are differentially expressed and incorporated into chromatin during differentiation and reprogramming to pluripotency. *J. Biol. Chem.* **286**, 35 347–35 357. (doi:10.1074/jbc.M111.281923)
83. Simonsson S, Gurdon J. 2004 DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei. *Nat. Cell Biol.* **6**, 984–990. (doi:10.1038/ncb1176)
84. Deng J *et al*. 2009 Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nat. Biotechnol.* **27**, 353–360. (doi:10.1038/nbt.1530)
85. Kim K *et al*. 2010 Epigenetic memory in induced pluripotent stem cells. *Nature* **467**, 285–290. (doi:10.1038/nature09342)

86. Lister R *et al*. 2009 Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462**, 315–322. (doi:10.1038/nature08514)
87. Bird A. 2002 DNA methylation patterns and epigenetic memory. *Genes Dev.* **16**, 6–21. (doi:10.1101/gad.947102)
88. De Carvalho DD, You JS, Jones PA. 2010 DNA methylation and cellular reprogramming. *Trends Cell Biol.* **20**, 609–617. (doi:10.1016/j.tcb.2010.08.003)
89. Laurent L *et al*. 2010 Dynamic changes in the human methylome during differentiation. *Genome Res.* **20**, 320–331. (doi:10.1101/gr.101907.109)
90. Eckhardt F *et al*. 2006 DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat. Genet.* **38**, 1378–1385. (doi:10.1038/ng1909)
91. Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, Schübeler D. 2007 Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.* **39**, 457–466. (doi:10.1038/ng1990)
92. Nagae G *et al*. 2011 Tissue-specific demethylation in CpG-poor promoters during cellular differentiation. *Hum. Mol. Genet.* **20**, 2710–2721. (doi:10.1093/hmg/ddr170)
93. Williams K, Christensen J, Pedersen MT, Johansen JV, Cloos PA, Rappsilber J, Helin K. 2011 TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. *Nature* **473**, 343–348. (doi:10.1038/nature10066)
94. Goll MG, Bestor TH. 2005 Eukaryotic cytosine methyltransferases. *Annu. Rev. Biochem.* **74**, 481–514. (doi:10.1146/annurev.biochem.74.010904.153721)
95. Chen T, Li E. 2004 Structure and function of eukaryotic DNA methyltransferases. *Curr. Top. Dev. Biol.* **60**, 55–89. (doi:10.1016/S0070-2153(04)60003-2)
96. Li E, Bestor TH, Jaenisch R. 1992 Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69**, 915–926. (doi:10.1016/0092-8674(92)90611-F)
97. Okano M, Bell DW, Haber DA, Li E. 1999 DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* **99**, 247–257. (doi:10.1016/S0092-8674(00)81656-6)
98. Dodge JE, Okano M, Dick F, Tsujimoto N, Chen T, Wang S, Ueda Y, Dyson N, Li E. 2005 Inactivation of Dnmt3b in mouse embryonic fibroblasts results in DNA hypomethylation, chromosomal instability, and spontaneous immortalization. *J. Biol. Chem.* **280**, 17 986–17 991. (doi:10.1074/jbc.M413246200)
99. Pawlak M, Jaenisch R. 2011 De novo DNA methylation by Dnmt3a and Dnmt3b is dispensable for nuclear reprogramming of somatic cells to a pluripotent state. *Genes Dev.* **25**, 1035–1040. (doi:10.1101/gad.2039011)
100. Jost JP, Siegmann M, Sun L, Leung R. 1995 Mechanisms of DNA demethylation in chicken embryos. Purification and properties of a 5-methylcytosine-DNA glycosylase. *J. Biol. Chem.* **270**, 9734–9739.
101. Vairapandi M, Duker NJ. 1993 Enzymic removal of 5-methylcytosine from DNA by a human DNA-glycosylase. *Nucleic Acids Res.* **21**, 5323–5327. (doi:10.1093/nar/21.23.5323)
102. Zhu B, Zheng Y, Angliker H, Schwarz S, Thiry S, Siegmann M, Jost J-P. 2000 5-Methylcytosine DNA glycosylase activity is also present in the human MBD4 (G/T mismatch glycosylase) and in a related avian sequence. *Nucleic Acids Res.* **28**, 4157–4165. (doi:10.1093/nar/28.21.4157)
103. Morgan HD, Dean W, Coker HA, Reik W, Petersen-Mahrt SK. 2004 Activation-induced cytidine deaminase deaminates 5-methylcytosine in DNA and is expressed in pluripotent tissues: implications for epigenetic reprogramming. *J. Biol. Chem.* **279**, 52 353–52 360. (doi:10.1074/jbc.M407695200)
104. Barreto G *et al*. 2007 Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. *Nature* **445**, 671–675. (doi:10.1038/nature05515)
105. Bhattacharya SK, Ramchandani S, Cervoni N, Szyf M. 1999 A mammalian protein with specific demethylase activity for mCpG DNA. *Nature* **397**, 579–583. (doi:10.1038/17533)
106. Wade PA, Gegonne A, Jones PL, Ballestar E, Aubry F, Wolffe AP. 1999 Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. *Nat. Genet.* **23**, 62–66. (doi:10.1038/12664)
107. Santos F, Hendrich B, Reik W, Dean W. 2002 Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev. Biol.* **241**, 172–182. (doi:10.1006/dbio.2001.0501)
108. Millar CB *et al*. 2002 Enhanced CpG mutability and tumorigenesis in MBD4-deficient mice. *Science* **297**, 403–405. (doi:10.1126/science.1073354)
109. Wong E *et al*. 2002 Mbd4 inactivation increases C⟶T transition mutations and promotes gastrointestinal tumor formation. *Proc. Natl Acad. Sci. USA* **99**, 14 937–14 942. (doi:10.1073/pnas.232579299)
110. Jin SG, Guo C, Pfeifer GP. 2008 GADD45A does not promote DNA demethylation. *PLoS Genet.* **4**, e1000013. (doi:10.1371/journal.pgen.1000013)
111. Ng HH, Zhang Y, Hendrich B, Johnson CA, Turner BM, Erdjument-Bromage H, Tempst P, Reinberg D, Bird A 1999 MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nat. Genet.* **23**, 58–61. (doi:10.1038/12659)
112. Rai K, Jafri IF, Chidester S, James SR, Karpf AR, Cairns BR, Jones DA. 2010 Dnmt3 and G9a cooperate for tissue-specific development in zebrafish. *J. Biol. Chem.* **285**, 4110–4121. (doi:10.1074/jbc.M109.073676)
113. Saitou M, Kagiwada S, Kurimoto K. 2012 Epigenetic reprogramming in mouse pre-implantation development and primordial germ cells. *Development* **139**, 15–31. (doi:10.1242/dev.050849)
114. Kriaucionis S, Heintz N. 2009 The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* **324**, 929–930. (doi:10.1126/science.1169786)
115. Tahiliani M *et al*. 2009 Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324**, 930–935. (doi:10.1126/science.1170116)
116. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. 2010 Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* **466**, 1129–1133. (doi:10.1038/nature09303)
117. Globisch D *et al*. 2010 Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. *PloS ONE* **5**, e15367. (doi:10.1371/journal.pone.0015367)
118. Song CX *et al*. 2011 Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. *Nat. Biotechnol.* **29**, 68–72. (doi:10.1038/nbt.1732)
119. Koh KP *et al*. 2011 Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. *Cell Stem Cell* **8**, 200–213. (doi:10.1016/j.stem.2011.01.008)
120. Ficz G, Branco MR, Seisenberger S, Santos F, Krueger F, Hore TA, Marques CJ, Andrews S, Reik W. 2011 Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation. *Nature* **473**, 398–402. (doi:10.1038/nature10008)
121. Freudenberg JM *et al*. 2011 Acute depletion of Tet1-dependent 5-hydroxymethylcytosine levels impairs LIF/Stat3 signaling and results in loss of embryonic stem cell identity. *Nucleic Acids Res.* **40**, 3364–3377. (doi:10.1093/nar/gkr1253)
122. Wu H, D'Alessio AC, Ito S, Xia K, Wang Z, Cui K, Zhao K, Eve Sun Y, Zhang Y. 2011 Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells. *Nature* **473**, 389–393. (doi:10.1038/nature09934)
123. Pastor WA *et al*. 2011 Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. *Nature* **473**, 394–397. (doi:10.1038/nature10102)
124. Yildirim O, Li R, Hung JH, Chen PB, Dong X, Ee LS, Weng Z, Rando OJ, Fazzio TG. 2011 Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells. *Cell* **147**, 1498–1510. (doi:10.1016/j.cell.2011.11.054)
125. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, He C, Zhang Y. 2011 Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* **333**, 1300–1303. (doi:10.1126/science.1210597)
126. He YF *et al*. 2011 Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science* **333**, 1303–1307. (doi:10.1126/science.1210944)
127. Dawlaty MM *et al*. 2011 Tet1 is dispensable for maintaining pluripotency and its loss is compatible with embryonic and postnatal development. *Cell Stem Cell* **9**, 166–175. (doi:10.1016/j.stem.2011.07.010)
128. de la Serna IL, Ohkawa Y, Imbalzano AN. 2006 Chromatin remodelling in mammalian

differentiation: lessons from ATP-dependent remodellers. *Nat. Rev. Genet.* **7**, 461–473. (doi:10.1038/nrg1882)
129. Gaspar-Maia A, Alajem A, Meshorer E, Ramalho-Santos M. 2011 Open chromatin in pluripotency and reprogramming. *Nat. Rev. Mol. Cell Biol.* **12**, 36–47. (doi:10.1038/nrm3036)
130. Clapier CR, Cairns BR. 2009 The biology of chromatin remodeling complexes. *Annu. Rev. Biochem.* **78**, 273–304. (doi:10.1146/annurev.biochem.77.062706.153223)
131. Ko M, Sohn DH, Chung H, Seong RH. 2008 Chromatin remodeling, development and disease. *Mutat. Res.* **647**, 59–67. (doi:10.1016/j.mrfmmm.2008.08.004)
132. Wang GG, Allis CD, Chi P. 2007 Chromatin remodeling and cancer. ATP-dependent chromatin remodeling. *Trends Mol. Med.* **13**, 373–380. (doi:10.1016/j.molmed.2007.07.004)
133. Kidder BL, Palmer S, Knott JG. 2009 SWI/SNF-Brg1 regulates self-renewal and occupies core pluripotency-related genes in embryonic stem cells. *Stem Cells* **27**, 317–328. (doi:10.1634/stemcells.2008-0710)
134. Lessard JA, Crabtree GR. 2010 Chromatin regulatory mechanisms in pluripotency. *Annu. Rev. Cell Dev. Biol.* **26**, 503–532. (doi:10.1146/annurev-cellbio-051809-102012)
135. Gao X, Tate P, Hu P, Tjian R, Skarnes WC, Wang Z. 2008 ES cell pluripotency and germ-layer formation require the SWI/SNF chromatin remodeling component BAF250a. *Proc. Natl Acad. Sci. USA* **105**, 6656–6661. (doi:10.1073/pnas.0801802105)
136. Kaeser MD, Aslanian A, Dong MQ, Yates 3rd JR, Emerson BM. 2008 BRD7, a novel PBAF-specific SWI/SNF subunit, is required for target gene activation and repression in embryonic stem cells. *J. Biol. Chem.* **283**, 32 254–32 263. (doi:10.1074/jbc.M806061200)
137. Ho L *et al*. 2009 An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. *Proc. Natl Acad. Sci. USA* **106**, 5181–5186. (doi:10.1073/pnas.0812889106)
138. Singhal N, Graumann J, Wu G, Arauzo-Bravo MJ, Han DW, Greber B, Gentile L, Mann M, Schöler HR. 2010 Chromatin-remodeling components of the BAF complex facilitate reprogramming. *Cell* **141**, 943–955. (doi:10.1016/j.cell.2010.04.037)
139. Ho L, Miller EL, Ronan JL, Ho WQ, Jothi R, Crabtree GR. 2011 esBAF facilitates pluripotency by conditioning the genome for LIF/STAT3 signalling and by regulating polycomb function. *Nat. Cell Biol.* **13**, 903–913. (doi:10.1038/ncb2285)
140. Kaji K, Caballero IM, MacLeod R, Nichols J, Wilson VA, Hendrich B. 2006 The NuRD component Mbd3 is required for pluripotency of embryonic stem cells. *Nat. Cell Biol.* **8**, 285–292. (doi:10.1038/ncb1372)
141. Marfella CG, Ohkawa Y, Coles AH, Garlick DS, Jones SN, Imbalzano AN. 2006 Mutation of the SNF2 family member Chd2 affects mouse development and survival. *J. Cell. Physiol.* **209**, 162–171. (doi:10.1002/jcp.20718)
142. Hurd EA, Capers PL, Blauwkamp MN, Adams ME, Raphael Y, Poucher HK, Martin DM. 2007 Loss of Chd7 function in gene-trapped reporter mice is embryonic lethal and associated with severe defects in multiple developing tissues. *Mamm. Genome: Official J. Int. Mamm. Genome Soc.* **18**, 94–104. (doi:10.1007/s00335-006-0107-6)
143. Nishiyama M *et al*. 2009 CHD8 suppresses p53-mediated apoptosis through histone H1 recruitment during early embryogenesis. *Nat. Cell Biol.* **11**, 172–182. (doi:10.1038/ncb1831)
144. Gaspar-Maia A *et al*. 2009 Chd1 regulates open chromatin and pluripotency of embryonic stem cells. *Nature* **460**, 863–868. (doi:10.1038/nature08212)
145. Schnetz MP *et al*. 2009 Genomic distribution of CHD7 on chromatin tracks H3K4 methylation patterns. *Genome Res.* **19**, 590–601. (doi:10.1101/gr.086983.108)
146. Zhu D, Fang J, Li Y, Zhang J. 2009 Mbd3, a component of NuRD/Mi-2 complex, helps maintain pluripotency of mouse embryonic stem cells by repressing trophectoderm differentiation. *PloS ONE* **4**, e7684. (doi:10.1371/journal.pone.0007684)
147. Reynolds N, Salmon-Divon M, Dvinge H, Hynes-Allen A, Balasooriya G, Leaford D, Behrens A, Bertone P, Hendrich B. 2011 NuRD-mediated deacetylation of H3K27 facilitates recruitment of Polycomb Repressive Complex 2 to direct gene repression. *EMBO J* **31**, 593–605. (doi:10.1038/emboj.2011.431)
148. McDonel P, Costello I, Hendrich B. 2009 Keeping things quiet: roles of NuRD and Sin3 co-repressor complexes during mammalian development. *Int. J. Biochem. Cell Biol.* **41**, 108–116. (doi:10.1016/j.biocel.2008.07.022)
149. Dovey OM, Foster CT, Cowley SM. 2010 Histone deacetylase 1 (HDAC1), but not HDAC2, controls embryonic stem cell differentiation. *Proc. Natl Acad. Sci. USA* **107**, 8242–8247. (doi:10.1073/pnas.1000478107)
150. Guan JS *et al*. 2009 HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature* **459**, 55–60. (doi:10.1038/nature07925)
151. Montgomery RL, Davis CA, Potthoff MJ, Haberland M, Fielitz J, Qi X, Hill JA, Richardson JA, Olson EN. 2007 Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. *Genes Dev.* **21**, 1790–1802. (doi:10.1101/gad.1563807)
152. Zimmermann S, Kiefer F, Prudenziati M, Spiller C, Hansen J, Floss T, Wurst W, Minucci S, Gottlicher M. 2007 Reduced body size and decreased intestinal tumor rates in HDAC2-mutant mice. *Cancer Res.* **67**, 9047–9054. (doi:10.1158/0008-5472.CAN-07-0312)
153. Trivedi CM *et al*. 2007 Hdac2 regulates the cardiac hypertrophic response by modulating Gsk3 beta activity. *Nat. Med.* **13**, 324–331. (doi:10.1038/nm1552)
154. Landry J *et al*. 2008 Essential role of chromatin remodeling protein Bptf in early mouse embryos and embryonic stem cells. *PLoS Genet.* **4**, e1000241. (doi:10.1371/journal.pgen.1000241)
155. Cremer T, Cremer C. 2001 Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat. Rev. Genet.* **2**, 292–301. (doi:10.1038/35066075)
156. Loden M, van Steensel B. 2005 Whole-genome views of chromatin structure. *Chromosome Res.: Int. J. Mol. Supramol. Evol. Aspects Chromosome Biol.* **13**, 289–298. (doi:10.1007/s10577-005-2166-z)
157. Siersbaek R, Nielsen R, Mandrup S. 2011 Transcriptional networks and chromatin remodeling controlling adipogenesis. *Trends Endocrinol. Metab.* **23**, 56–64. (doi:10.1016/j.tem.2011.10.001)
158. Francastel C, Schubeler D, Martin DI, Groudine M. 2000 Nuclear compartmentalization and gene activity. *Nat. Rev. Mol. Cell Biol.* **1**, 137–143. (doi:10.1038/35040083)
159. Arney KL, Fisher AG. 2004 Epigenetic aspects of differentiation. *J. Cell Sci.* **117**, 4355–4363. (doi:10.1242/jcs.01390)
160. Carey BW *et al*. 2011 Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. *Cell Stem Cell* **9**, 588–598. (doi:10.1016/j.stem.2011.11.003)
161. Inui M, Martello G, Piccolo S. 2010 MicroRNA control of signal transduction. *Nat. Rev. Mol. Cell Biol.* **11**, 252–263. (doi:10.1038/nrn2804)
162. Marson A *et al*. 2008 Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. *Cell* **134**, 521–533. (doi:10.1016/j.cell.2008.07.020)
163. Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R. 2007 DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat. Genet.* **39**, 380–385. (doi:10.1038/ng1969)
164. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K. 2005 Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev.* **19**, 489–501. (doi:10.1101/gad.1248505)
165. Murchison EP, Partridge JF, Tam OH, Cheloufi S, Hannon GJ. 2005 Characterization of dicer-deficient murine embryonic stem cells. *Proc. Natl Acad. Sci. USA* **102**, 12 135–12 140. (doi:10.1073/pnas.0505479102)
166. Melton C, Judson RL, Blelloch R. 2010 Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature* **463**, 621–626. (doi:10.1038/nature08725)
167. Lakshmipathy U, Love B, Goff LA, Jornsten R, Graichen R, Hart RP, Chesnut JD. 2007 MicroRNA expression pattern of undifferentiated and differentiated human embryonic stem cells. *Stem Cells Dev.* **16**, 1003–1016. (doi:10.1089/scd.2007.0026)
168. Viswanathan SR, Daley GQ, Gregory RI. 2008 Selective blockade of microRNA processing by Lin28. *Science* **320**, 97–100. (doi:10.1126/science.1154040)
169. Yu J *et al*. 2007 Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917–1920. (doi:10.1126/science.1151526)
170. Sampson VB, Rong NH, Han J, Yang Q, Aris V, Soteropoulos P, Petrelli NJ, Dunn SP, Krueger LJ.

2007 MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. *Cancer Res.* **67**, 9762–9770. (doi:10.1158/0008-5472.CAN-07-2462)

171. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, Dang CV, Thomas-Tikhonenko A, Mendell JT. 2008 Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat. Genet.* **40**, 43–50. (doi:10.1038/ng.2007.30)
172. Stadtfeld M, Apostolou E, Akutsu H, Fukuda A, Follett P, Natesan S, Kono T, Shioda T, Hochedlinger K. 2010 Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* **465**, 175–181. (doi:10.1038/nature09017)
173. Okamoto I, Otte AP, Allis CD, Reinberg D, Heard E. 2004 Epigenetic dynamics of imprinted X inactivation during early mouse development. *Science* **303**, 644–649. (doi:10.1126/science.1092727)
174. Payer B, Lee JT. 2008 X chromosome dosage compensation: how mammals keep the balance. *Annu. Rev. Genet.* **42**, 733–772. (doi:10.1146/annurev.genet.42.110807.091711)
175. Maherali N *et al*. 2007 Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* **1**, 55–70. (doi:10.1016/j.stem.2007.05.014)
176. Tchieu J *et al*. 2010 Female human iPSCs retain an inactive X chromosome. *Cell Stem Cell* **7**, 329–342. (doi:10.1016/j.stem.2010.06.024)
177. Nichols J, Smith A. 2009 Naive and primed pluripotent states. *Cell Stem Cell* **4**, 487–492. (doi:10.1016/j.stem.2009.05.015)
178. Okamoto I *et al*. 2011 Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development. *Nature* **472**, 370–374. (doi:10.1038/nature09872)
179. Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, Smith A. 2008 Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biol.* **6**, e253. (doi:10.1371/journal.pbio.0060253)
180. Hanna J *et al*. 2010 Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. *Proc. Natl Acad. Sci. USA* **107**, 9222–9227. (doi:10.1073/pnas.1004584107)
181. Payer B, Lee JT, Namekawa SH. 2011 X-inactivation and X-reactivation: epigenetic hallmarks of mammalian reproduction and pluripotent stem cells. *Hum. Genet.* **130**, 265–280. (doi:10.1007/s00439-011-1024-7)
182. Plath K, Lowry WE. 2011 Progress in understanding reprogramming to the induced pluripotent state. *Nat. Rev. Genet.* **12**, 253–265. (doi:10.1038/nrg2955)
183. Doi A *et al*. 2009 Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat. Genet.* **41**, 1350–1353. (doi:10.1038/ng.471)