Chromatin regulation landscape of embryonic stem cell identity

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Synopsis
ES cells (embryonic stem cells) derived from the ICM (inner cell mass) of blastocysts are pluripotent and are capable of giving rise to most cell types. The ES cell identity is mainly maintained by the Oct4 (octamer-binding transcription factor 4) and Nanog transcriptional networks. Recently, a tremendous amount of work has focused on deciphering how ES cell identity is regulated epigenetically. It has been shown that histone methylation/demethylation, histone acetylation/deacetylation, histone variants and chromatin remodelling play crucial roles in ES cell maintenance and differentiation. Moreover, perturbation of those chromatin regulators results in loss of ES cell identity or aberrant differentiation. Therefore, it is important to fully understand the chromatin regulation landscape of ES cells. The knowledge gained will help us to harness the unique characteristics of ES cells for stem cell-related therapy and regenerative medicine. In the present review, we will discuss recent proceedings that provide novel insights into chromatin regulation of ES cell identity.

Key words: chromatin, chromatin remodelling, differentiation, embryonic stem cell (ES cell), embryonic stem cell identity, histone modification

INTRODUCTION

In mammals, the totipotent zygote passes through the 2-, 4- and 8-cell stages of development, before reaching the morula (16-cell) stage and, finally, the blastocyst stage when uterine implantation occurs. In the pre-implantation mouse embryo, the first cell fate decision occurs when the embryonic cells give rise to two distinct lineages: the pluripotent ICM (inner cell mass) and the differentiated trophectoderm which envelops the ICM [1]. The ICM will subsequently develop into the epiblast and hypoblast (primitive endoderm) [1]. The ICM and epiblast cells that form the embryo are considered pluripotent as they have the ability to develop into three germ layers: ectoderm, mesoderm and endoderm [2].

ES cells (embryonic stem cells) derived from the ICM can be cultured indefinitely in vitro in an ES-specific medium. They are characterized by their ability to self-renew and by their potential to differentiate into all the tissue types of the adult body [3,4]. Strikingly, ES cells remain relatively consistent in their ability to re-enter embryogenesis and can produce viable chimaeras after being injected into blastocysts [5].

Due to their unique properties, ES cells provide a good in vitro system to better understand the factors and pathways that determine pluripotency and differentiation [2,6]. In addition, ES cells show great potential for treating degenerative diseases such as diabetes and Parkinson’s disease [7], especially after the discovery that ES cells can be obtained from human blastocysts [8]. Due to the importance of ES cell research, much effort has been put into this intriguing field. One of the fundamental questions of ES cell research is how ES cell identity is regulated. In this review, we will briefly review the extrinsic and intrinsic factors that are responsible for ES cell identity. We will focus on our current knowledge of the functions and molecular mechanism of chromatin modification in maintaining ES cell identity.

Abbreviations used: BAF, Brg/Brahma-associated factor; BMP, bone morphogenetic protein; Brg1, Brahma-related gene 1; Brm, Brahma; Carm, co-activator-associated arginine methyltransferase; CHD, chromodomains; ChHf, chromatin immunoprecipitation; ChIP-seq, ChIP-sequencing; ES cell, embryonic stem cell; Ezh, enhancer of zeste homologue; H3K4me3, trimethylated H3K4; HAT, histone acetyltransferase; H3K27me3, histone H3 lysine 27 trimethylated; HDAC, histone deacetylase; HDMases, histone demethylases; HMTase, histone methyltransferase; HP1, heterochromatin protein 1; ICM, inner cell mass; LIF, leukaemia inhibitory factor; Mbd3, methyl-CpG-binding domain protein 3; MEF, mouse embryonic fibroblast; NPC, neural progenitor cell; NuRD, nucleosome remodelling and deacetylase; Oct4, octamer-binding transcription factor 4; Pdgf, polycystic group; Pu2, polycomb-like 2; PRC, polycomb repressor complex; RNAP, RNA polymerase; SalI, SalI-like protein 4; Snf2, switch/sucrose non-fermentable; Sox, SRY (sex-determining region Y)-related HMG (high-mobility group) box protein; Stat3, signal transducer and activator of transcription 3.

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EXTRINSIC AND TRANSCRIPTIONAL REGULATORS OF ES CELL IDENTITY

The LIF (leukaemia inhibitory factor)/Stat3 (signal transducer and activator of transcription 3) signalling is essential for murine ES cell culture. LIF acts via the gp130 (glycoprotein 130) receptor, which in turns results in the JAKs (Janus kinases)-mediated activation of transcription factor Stat3 [9]. The activated Stat3 is translocated into the nucleus, where it subsequently activates the transcription of target genes that are essential for ES cell self-renewal [9]. In addition, BMPs (bone morphogenetic proteins) in fetal calf serum are also believed to act in combination with LIF and play a role in sustaining pluripotency in ES cells [10]. The binding of BMP to its receptors stimulates the phosphorylation of Smad1 and promotes Id (inhibitor of differentiation) gene expression, which regulates ES cell pluripotency and self-renewal [10]. However, it has been reported that the ground state of ES pluripotency and self-renewal can be sustained by simply blocking differentiation-inducing signals, Fgf (fibroblast growth factor), Erk (extracellular-signal-regulated kinase) and Gsk3 (glycogen synthase kinase 3) [11]. Consistently, this is also seen in blastocyst ICM, whereby the primitive endoderm may be blocked with different combinations of these inhibitors, although this may be reversible [12,13]. These suggest that the extrinsic signals are dispensable for the ground state of ES pluripotency and self-renewal [14].

The genetic control of ES identity has been extensively studied [15]. The transcription factors Oct4 (octamer-binding protein 4) and Nanog are core regulators of ES cell pluripotency. Oct4 is a POU domain transcription factor. Loss of Oct4 directs ES cells into trophoderm lineages [16]. However, overexpression of Oct4 in ES cells causes differentiation towards the endoderm and mesoderm lineages [17]. This implies that Oct4 levels have to be precise in order to regulate pluripotency and that the LIF-induced pathway has to be active at the same time [17]. Nanog, a homeodomain protein, is essential for ES cell pluripotency [18–20]. When overexpressed, it has the ability to maintain pluripotency in ES cells in the absence of LIF or BMP [18]. Deficiency of Nanog often results in differentiation of ES cells into primitive endoderm [18,19,21]. The SRY (sex-determining region Y)-related HMG (high-mobility group)-box protein, Sox2, interacts with Oct4 to regulate the expression of target genes [22,23]. Sall4 (Sal-like protein 4) regulates Oct4 and many other pluripotency genes [24,25]. Sall4 and Nanog form the auto-repression and network feedback motifs in the Nanog/Sall4 circuit, similar to that of the Oct4/Sox2 circuit [26].

Besides Oct4, Sox2, Nanog and Sall4, many other transcription factors, such as Esr2b [27,28] and Klf4 (Krüppel-like factor 4) [29,30], have been shown to be important for ES cell identity [31,32]. In fact, some of these transcription factors have the ability to induce pluripotency in terminally differentiated cells [33,34].

ChIP (chromatin immunoprecipitation)-on-chip and ChIP-seq (ChIP sequencing) studies have demonstrated that Oct4, Sox2 and Nanog activate many downstream pluripotency genes (including themselves), while silencing development-promoting genes [35–38]. In addition, Oct4 and Nanog also interact with a set of partners to maintain ES cell identity [39–41]. These core regulators, together with their partners and downstream factors, cooperate to form a complex transcriptional network containing diverse feed-forward and auto-regulatory loops, which contribute largely to the regulation of ES cell identity.

THE UNIQUE CHROMATIN STATE OF ES CELLS

Epigenetic regulation including DNA methylation, post-translational modification of histones, chromatin remodelling and non-coding RNA, can alter gene expression activity without a change in the nucleotide sequence of DNA [42]. A number of loss-of-gain-of function studies have implicated chromatin regulators in pivotal roles in embryonic development and maintenance of ES cell identity [15,43]. Here, we focus on recent findings on how chromatin regulations control ES cell identity.

One of the striking properties of ES cells is that development-promoting genes are steadily silenced, although the cells are poised to be driven into the differentiated state. With its distinct transcriptome profile of activated pluripotency genes and suppressed lineage genes, the ES chromatin state provides an important environment for ES cell identity.

A recent single-cell mRNA-seq study demonstrated that during the course of ICM outgrowth towards ES cells, the repressive epigenetic regulators such as HDACs (histone deacetylases), H3K9 and H4K20 methyltransferase increase significantly, while a large number of active epigenetic regulators such as p300, H3K9 and H3K27 demethylases are down-regulated [44]. This suggests that ES cells compared with their embryonic counterpart, the ICM, are maintained in a more repressive epigenetic status.

However, when compared with lineage-committed cells, ES cells generally display an open and decondensed chromatin that is transcriptionally more accessible, with the high levels of histone acetylation and H3K4 methylation to sustain pluripotency [45,46]. Further, the hyperdynamic binding of chromatin structural proteins and bivalent histone modifications may allow ES cells to be more responsive and permissive when they differentiate [46,47]. A further genome-wide chromatin-state study proposed that H3K4 and H3K27 methylation might reflect developmental commitment and potential [48]. Indeed, recent studies demonstrate that lineage-committed cells are characterized by significantly expanded repressive chromatin domains, which selectively affect pluripotency and developmental genes [49,50]. Upon differentiation, ES cells demonstrate increased transcriptionally inactive heterochromatin regions with increased H3K9 and H3K27 methylation [46,49,50]. This unique chromatin state is also distinct from other embryo-derived stem cells such as TS cells (trophoblast stem cells) and XEN (extra-embryonic endoderm stem) cells in which bivalent domains are not common [51].
HISTONE LYSINE METHYLATION

H3K4me3 (trimethylated H3K4) catalysed by Trithorax is a hallmark of active promoters and is enriched in the promoters of core transcription factors, Oct4, Sox2 and Nanog, which contribute to the self-renewal properties of ES cells [52,53]. Interestingly, the repressive chromatin mark H3K27me3 is often found to harbour small regions of the activation mark H3K4me3 [45,47,48]. These ‘bivalent’ domains are associated with the highly conserved non-coding elements and occur frequently at Oct4, Sox2 and Nanog target genes in mouse ES cells [47]. Importantly, they are found largely in the developmentally important genes that are silenced in ES cells, but are poised to be activated upon differentiation [47]. The fate of bivalent domains was impressively explored by a subsequent ChIP-seq study [48]. In this study, by mapping H3K4me3, H3K27me3 and other histone marks using high-resolution sequencing technology, Mikkelsen et al. [48] demonstrated that a large number of promoters (~2500) are associated with bivalent domains in mouse ES cells, whereas much fewer bivalent domains are present in more committed mouse NPCs (neural progenitor cells) and MEFs (mouse embryonic fibroblasts). Interestingly, 46 % of the bivalent marks in ES cells resolve to H3K4me3 alone and the associated genes exhibit increased expression in NPCs. The other half either resolves to H3K27me3 alone or lose both marks, or remain bivalent, all of which are associated with no or low transcription [48]. In MEFs, approximately half of the regions remain bivalent, which may be related to their less differentiated state [48]. These studies suggested that chromatin bivalency generally represses transcription, but also provides a balanced machinery to keep developmental genes poised to be activated or continuously silenced upon distinct differential signals.

In ES cells, H3K27me3 is catalysed by PRC2 (polycomb repressor complex 2) that consists of three core proteins, Eed, Suz12 (suppressor of zeste 12 protein homologue) and Ezh2 (enhancer of zeste homologue 2), and has been found at the promoter regions of repressed developmental genes [45,54]. Ezh1, a homologue of Ezh2, is an alternative H3K27 methyltransferase and complements gene suppression mediated by Ezh2 [55]. Disruption of H3K27me3 leads to de-repression or even premature expression of these genes [45,54]. Unexpectedly, ES cells lacking the function of either PRC1 or PRC2 are still pluripotent and maintain the ability to differentiate, suggesting that polycomb complexes act redundantly to inhibit genes in ES cells [56,57]. Nonetheless, PRC2 is required for ES cells to direct the reprogramming of differentiated cells towards pluripotency [58].

Recently, it was reported that Jarid2, a Jumonji C domain-containing protein devoid of detectable demethylase activity, can bind to DNA and co-localize with Ezh2, resulting in increased level of H3K27 methylation [59–63]. Moreover, PRC2 activity is largely reduced in ES cells lacking Jarid2 [59–63]. These findings highlight the importance of Jarid2 in recruiting the PRC2 complex to promoters of target genes. Besides Jarid2, it has been shown that Pcl2 (polycomb-like 2) is associated with PRC2 complex and co-occupies a large number of targets with PRC2. Depletion of Pcl2 results in heightened self-renewal and defects in differentiation [64].

Another epigenetic mark that has been associated with transcriptional silencing and heterochromatin in ES cells is methylation of H3K9 [48–50]. G9a, Glp and Eset are the major HMTases (histone methyltransferases) of H3K9 that target the euchromatin regions of pluripotent ES cells. Knockout of these HMTases leads to embryonic lethality [15]. Importantly, Eset, a histone H3K9 methyltransferase, maintains ES pluripotency by interacting with Oct4 and suppressing trophectoderm genes Cdx2. Loss of Eset results in differentiation into trophoderm [65–67].

On the other hand, HDMases (histone demethylases) also play a critical role in regulating pluripotency in ES cells. Oct4 is found to positively regulate the expression of two HDMases of the Jmjd family [JHDM (JmJD domain-containing histone demethylase)], namely Jmjd1a and Jmjd2c [36,68]. Jmjd1a demethylates H3K9 at the promoter regions and regulates the expression of pluripotency-associated genes such as Tcl1 [68]. Jmjd2c positively regulates Nanog by preventing the recruitment of repressor protein HP1 (heterochromatin protein 1) and Kap1 to the Nanog promoter region. Absence of these HDMases leads to ES cell differentiation, indicating their importance in sustaining ES identity [68]. In addition, an H3K4 demethylase, Jarid1a (Rhb2), was found to bind to PRC2 and hence be associated with a large number of PRC2 target genes, suggesting that H3K4 demethylation is necessary for gene repression mediated by PRC2 [69].

HISTONE ACETYLATION

Histone acetylation is positively correlated to active transcription [70]. The ES cell genome is generally associated with the high levels of histone acetylation and H3K4me3. As pluripotency is lost when ES cells undergo differentiation, the levels of histone acetylation for both H3 and H4 decrease as well [71]. This is mainly due to the loss of hyperdynamic binding of structural chromatin proteins [46]. Using ChIP assays, studies have demonstrated that the enhancer/promoter region of Oct4 is highly acetylated in ES cells [72]. This is consistent with the observation in the ICM, where AcH4 (acetylation of histone H4) is found to be enriched at the promoter regions of Nanog and Pou5f1 [73].

Transcription factors are known to interact with HATs (histone acetyltransferases) for gene activation. HATs are generally divided into three main families: GNAT (Gcn5-related N-acetyltransferases), MYST (MOZ, Ybf2/Sas3, Sas2 and Tip60) and CBP (cAMP-response-element-binding protein-binding protein)/p300 [74]. The histone acetylase and co-activator p300 were found to co-occupy Oct4-Nanog-Sox2 genomic sites in ES cells [37]. p300 might be recruited by the core transcription factors and serve as a gene activator [37]. Conversely, p300 also regulates Nanog levels by acetylation of the Nanog distal enhancer and p300 is essential for appropriate differentiation of ES cells [75].
It is remarkable that H3K56 acetylation, which unlike most of the other histone modifications does not occur at the N-terminal of histones, overlays many active target genes with core transcription factors OCT4, SOX2 and NANOG in human ES cells [76]. Upon differentiation, H3K56 acetylation marks developmental genes. Thus, H3K56 acetylation may define differences between human ES cells and somatic cells [76]. H3K56 acetylation is mediated by CBP/p300 and also correlated to DNA repair, cell growth and cancer [77]. It is noteworthy that an elegant RNAi (RNA interference) screen has identified a histone acetylation and nucleosome remodelling complex, Tip60-p400, as an important regulator of ES cell identity [78]. Tip60-p400 overlaps targets with Nanog, promotes histone H4 acetylation in correlation with H3K4 methylation and thus is necessary to maintain ES cell identity [78].

Apart from HATs, HDACs are equally important. Inhibition of HDAC activity is known to disrupt the formation of compact heterochromatin regions and affect cell differentiation [71]. However, it seems that the deletion of Hdac1 or Hdac2 does not affect ES cell self-renewal [79]. Nevertheless lack of Hdac1 increases H3K56 acetylation and enhances EB (embryonic body) differentiation [79]. Although HDACs are redundant for ES cell identity, Oct4 and Nanog can interact with a unique Hdac1/2- and Mta1/2 (metastasis-associated protein 1/2)-containing complex and co-occupy Nanog target genes [80]. Another study has shown that the mSin3a-HDAC complex interacts with Sox2, binds to the Nanog promoter and positively regulates Nanog in ES cells [81]. These studies reflect the important role of HDAC activity in suppressing lineage genes in ES cells.

**OTHER HISTONE MODIFICATIONS**

**Histone arginine methylation**

Arginine methylation of histone proteins has been implicated with cell fate determination and pluripotency in pre-implantation mouse embryos [82]. High methylation levels of histone arginine methylation mediated by Carm1 (co-activator-associated arginine methyltransferase 1) increases Nanog levels and directs cell progeny into the pluripotent ICM [82]. Consistently, in ES cells, Carm1, also known as Prmt4, is essential for the pluripotency and self-renewal process [83]. Depletion of CARM1 in ES cells reduces H3R17 and H3R26 methylation levels on Oct4 and Nanog promoters, leading to down-regulation of pluripotency genes and subsequent differentiation [83]. These findings highlight the importance of histone arginine methylation in early embryonic development and ES cells.

**Histone H2A ubiquitination**

Recent studies by Stock et al. [84] demonstrated that RNAP (RNA polymerase) complexes present at the poised genes in ES cells are enforced by the PRC1-mediated mono-ubiquitination of histone H2A. Depletion of Ring1A and Ring1B reduces the levels of H2A ubiquitination, accompanied by the release of the RNAP and rapidly de-represses the poised genes, indicating the importance of H2A ubiquitination in regulating the developmental genes upon differentiation [85]. The deletion of Ring1B causes the loss of several PcG (polycomb group) proteins and an aberrant differentiation potential [85]. In addition, PRC1 overlaps a subset of downstream genes with Oct4 and the suppression mediated by PRC1 is Oct4 dependent [86]. Interestingly, it has been suggested that the gene suppression function of Ring1B is due to chromatin compaction and direct transcription inhibition [87]. Thus, the function of Ring1B may not be dependent on its ubiquitination activity.

**Histone clipping**

Histone modification by proteolysis is a potential mode of transcriptional regulation during cell differentiation [88,89]. This mechanism involves proteolytic cleavage of the first 21 residues at the histone H3 N terminus. In mouse ES cells, cathepsin L from the family of cysteine proteases is responsible for this H3 cleavage activity [88]. Proteolysis of H3 by cathepsin L occurs during differentiation of ES cells and perhaps regulates the transcriptional state of developmental genes by clipping off their repressive marks [88], as observed in the yeast model [89].

**Histone variants**

Notably, recent genome-wide studies have shown that the histone variants are important regulators of ES cell identity. For example, histone H2AZ and the PcG protein Suz12 interdependently co-occupy a large number of promoters of developmentally important genes [90]. Although it is not required for ES pluripotency and self-renewal, histone H2AZ is necessary for appropriate differentiation [90]. Another histone variant histone H3.3 was found to correlate with markers of transcription and is enriched around the transcriptional start site of bivalent genes in ES cells [91]. It is of interest that ATRX (Alpha thalassaemia/mental retardation syndrome X-linked), an SWI2/SNF2 (switch/sucrose non-fermentable 2) family of chromatin remodelling proteins, is required for H3.3 to localize at telomeres so as to maintain ES telomere integrity [91–93]. Future studies could be conducted to provide new insights into functions of histone replacement.

**CHROMATIN REMODELLING**

Pluripotent ES cells are characterized by a hyperdynamic chromatin structure, and upon differentiation, the plasticity of the chromatin is altered into a more content state. To achieve this, the ATP-dependent chromatin remodelling enzymes utilize the energy from ATP hydrolysis to effectively shift nucleosome cores along the length of the DNA. This in turn modulates the DNA accessibility towards chromatin proteins or transcription factors [94]. Mammalian SWI/SNF complex, also known
Chromatin landscape of ES cell identity

Figure 1  Histone H3 methylation and acetylation are essential for ES cell identity
Amino acid sequence of mouse histone H3 is indicated. The modified residues are stated and the enzymes responsible for the modifications are listed. Methylation (ME) of Lys⁴ and Lys⁳⁶, Arg¹⁷ and Arg²⁶, as well as acetylation (AC) of Lys¹⁴, Lys¹⁸, Lys³⁶ and Lys⁵⁶ are associated with gene activation (green), whereas Lys⁹⁷ methylation is associated with gene suppression (red). Methylation on Lys⁹ or Lys²⁷ (orange) is associated with gene suppression, whereas acetylation on Lys⁹ or Lys²⁷ is associated with gene activation. Scissors represents the site of histone clipping by the enzyme cathepsin L (blue). Hexagons represent methyltransferases; ovals represent demethylases; diamonds represent acetyltransferases.

as BAFs (Brg/Brahma-associated factors), has been implicated in regulating ES cell identity and their capacity to differentiate [95]. This enzyme is a multi-subunit complex containing an ATPase, either Brg1 (Brahma-related gene 1) or Brm (Brahma), both with a bromodomain that preferentially interacts with acetylated histones [96]. Depletion of Brg1 ATPase and other BAFs, including Baf47 and Baf155, results in embryonic lethality [97–99]. In ES cells, knockdown of Brg1 leads to the loss of self-renewal, and cells differentiate into ectodermal and mesodermal lineages [100]. Proteomic studies reveal that, apart from Brg1, pluripotent ES cells are also characterized by high levels of Baf155 and Baf60a. However, the expression of Brm, Baf170 and Baf60c is minimal [101]. Brg1 is also essential in maintaining ES colony morphology [78] and has been implicated in reprogramming [102]. Brg1 has recently been found to interact with Nanog in order to regulate ES cell pluripotency [80]. Furthermore, Baf155 has been reported to promote increased repressive H3K27me3 marks at the Nanog promoter and is essential for heterochromatin formation and chromatin compaction during ES cell differentiation [103]. Furthermore, deletion of Baf250b reduces the ES cell proliferation rate and impairs the self-renewal capacity of undifferentiated ES cells. Thus, Baf250b is required to maintain the undifferentiated state of ES cells [104].

Another type of chromatin remodelling enzymes belongs to the CHD (chromodomain) family and contains an ATPase SNF2-like helicase domain [105]. There are a total of nine CHD proteins in the family, and some of them have been shown to play essential roles in regulating ES cells. CHD1 has previously been associated with active transcription [106]. Recently, studies in ES cells have revealed that Chd1 is bound by core transcription factors Oct4, Sox2 and Nanog, indicating that it is an important target in regulating ES cell identity [37]. Down-regulation of Chd1 results in decreased expression of Oct4 and also leads to a dramatic increase in expression of genes involved in neurogenesis. Hence, cells become prone to neural differentiation [107]. ES cells with depleted Chd1 have highly condensed heterochromatin structures that are enriched in H3K9me3 and HP1. Consequently, these cells lose their pluripotency. In addition, Chd1 is found to be important for efficient reprogramming of fibroblasts to the pluripotent state, indicating that it is an important factor in maintaining ES cell pluripotency [107]. Another member from the CHD family, Chd7, may also have a role in ES cell pluripotency. Disruption of Chd7 is found to cause embryonic lethality in early development [108]. Genome-wide analysis revealed that in ES cells, Chd7 binding is associated with all methylated forms of H3K4 and characterizes active gene expression [109]. Upon differentiation, Chd7 will be re-localized to the promoter regions of developmental genes [109]. Finally, the perturbing level of Chd4 (Mi-2β or NuRD (nucleosome remodelling and deacetylase) complex dysregulates haemopoietic stem cell self-renewal and lineage priming [110]. The importance of NuRD in ES cell identity was highlighted by the functions of Mbd3 (methyl-CpG-binding domain protein 3), one of the components of NuRD, since ES cells lacking Mbd3 fail to commit to developmental lineages [111].

CONCLUSIONS

ES cells are characterized by their unique features of pluripotency and their ability to self-renew indefinitely. Dynamic regulation of chromatin structure is an important mechanism for balancing the pluripotency and cell fate decision in ES cells. The importance of chromatin factors in sustaining ES cell identity is becoming increasingly clear over the period of time (Figure 1 and Table 1). Yet there are some fundamental unaddressed questions: how do extrinsic signals, transcription factors and chromatin...
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<td>Chromatin remodelling complex</td>
<td>Atrx</td>
<td>Interacts with histone H3.3 to regulate telomere chromatin in ES cells. Depletion leads to a high rate of spontaneous differentiation in ES cells and telomerase dysfunction.</td>
<td>[93]</td>
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<td></td>
<td>Baf250b</td>
<td>Maintains the normal proliferation and pluripotency in ES cells. Depletion causes defective self-renewal in ES cells.</td>
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<td>Chd1</td>
<td>Maintains the euchromatin status and pluripotency in ES cells. Deficiency results in neural differentiation.</td>
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<td>Chd7</td>
<td>Correlates with H3K4 methylation to mediate transcription. Depletion results in embryonic lethality.</td>
<td>[108,109]</td>
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<td>Brg1</td>
<td>Maintains the pluripotency by regulating gene expression. Deficiency results in differentiation, decreased self-renewal capacity and reduced expression of pluripotency genes.</td>
<td>[99,100]</td>
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<td>Baf155</td>
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<td>NuRD</td>
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<td>Snf5/Ini1</td>
<td>Facilitates the nucleosome assembly. Deficiency causes peri-implantation lethality.</td>
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<td>Histone clipping enzyme</td>
<td>Cathepsin L</td>
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<td>HDAC</td>
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<td>Tip60-p400</td>
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<td>Histone demethylase</td>
<td>Jarid1a</td>
<td>Interacts with PRC2 complex to repress the PRC2 target genes. Deficiency leads to cell differentiation.</td>
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<td>Jmjd1a</td>
<td>Binds and demethylates H3K9 at the promoter region of the pluripotency-related genes to regulate gene expression. Depletion leads to cell differentiation.</td>
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<td></td>
<td>Jmjd2c</td>
<td>Regulates Nanog expression by demethylating H3K9 at its promoter. Depletion results in the recruitment of repressor proteins HP1 and KAP1.</td>
<td>[68]</td>
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<td>Histone methyltransferase</td>
<td>Carm1</td>
<td>Promotes expression of key pluripotency genes and directs cells to the pluripotent ICM. Deficiency causes cell differentiation due to the down-regulation of pluripotency genes.</td>
<td>[82,83]</td>
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<td></td>
<td>Eset</td>
<td>Interacts with Oct4 to suppress trophectoderm gene Cdx2. Deficiency results in cell differentiation into trophectoderm.</td>
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<td>Ezh1</td>
<td>Completes Ezh2-mediated H3K27me3 at the developmental genes. Depletion de-represses the developmental genes.</td>
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<td>Histone variant</td>
<td>H2AZ</td>
<td>Co-operates with Suz12 to regulate developmental gene expression. Deficiency causes impairment in proper differentiation.</td>
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<td>H3.3</td>
<td>Interacts with Atrx to maintain ES telomere structural integrity. Depletion results in telomere dysfunction.</td>
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<td>Polycomb group protein</td>
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<td>Pcl2</td>
<td>Recruits PRC2 complex and represses members of the pluripotency network during differentiation. Depletion causes altered patterns of H3K27me3 and impairment in proper differentiation.</td>
<td>[64]</td>
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<td>Ubiquitin ligase complex</td>
<td>Ring1b</td>
<td>Represses the developmental genes. Deficiency de-represses the lineage genes, and results in defective gastrulation and early embryonic lethality.</td>
<td>[84–87]</td>
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</table>
modifications collaborate and become integrated into a fine-tuned network to maintain ES cell identity? How do chromatin-binding proteins and transcriptional machineries find histone modifications at specific loci? Finally, in the early embryos, how do cells establish histone marks in the ICM, erase the old histone marks and re-establish new histone marks rapidly when ICM cells differentiate? Nonetheless, with the assistance of advanced technology, such as ChIP-seq and high-resolution in vivo imaging, we expect the mechanism underlying chromatin regulation of ES cell identity to be revealed in greater detail in the near future.

ACKNOWLEDGEMENTS
We thank Eunice Lin and Alex Bruce for critical reading of the manuscript.

FUNDING
The authors’ work is supported by the National University of Singapore and the Singapore Ministry of Education.

REFERENCES


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Received 29 July 2010/31 August 2010; accepted 14 September 2010
Published on the Internet 29 October 2010, doi 10.1042/BSR20100089