Published in final edited form as:

Trends Pharmacol Sci. 2011 July ; 32(7): 394–401. doi:10.1016/j.tips.2011.03.002.

Stem cells and reprogramming: breaking the epigenetic barrier?

Yen-Sin Ang^{1,2,3,*}, Alexandre Gaspar-Maia^{1,4,*}, Ihor R. Lemischka^{1,2,3}, and Emily Bernstein^{1,4,5}

¹ Black Family Stem Cell Institute, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, New York, 10029 USA

² Department of Gene and Cell Medicine, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, New York, 10029 USA

³ Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, New York, 10029 USA

⁴ Department of Oncological Sciences, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, New York, 10029 USA

⁵ Department of Dermatology, Mount Sinai School of Medicine, 1425 Madison Avenue, New York, New York, 10029 USA

Abstract

Increasing evidence suggests that epigenetic regulation is key for the maintenance of the stem cell state. Chromatin is the physiological form of eukaryotic genomes and the substrate for epigenetic marking, including DNA methylation, post-translational modifications of histones, and the exchange of core histones with histone variants. The chromatin template undergoes significant reorganization during embryonic stem cell (ESC) differentiation and somatic cell reprogramming (SCR). Intriguingly, remodeling of the epigenome appears to be a crucial barrier that must be surmounted for efficient SCR. This area of research has gained significant attention due to the importance of ESCs in modeling and treating human disease. Here we review the epigenetic mechanisms that are critical for maintenance of the ESC state, ESC differentiation and SCR. We focus our attention on murine and human ESCs and induced pluripotent stem cells (iPSCs), and highlight pharmacological approaches used to study or manipulate cell fate where relevant.

Introduction

Embryonic stem cells (ESCs), derived from the inner cell mass of blastocyst stage embryos, possess the ability not only to self-renew but also to form all cell types in the body [1]. Therefore, ESCs hold enormous therapeutic potential for regenerative medicine. ESCs and their derivatives offer unprecedented tools to improve our understanding of complex diseases, develop innovative pharmacological compounds and, ultimately, patient-specific therapies. In recent years, advances in somatic cell reprogramming (SCR) have been revolutionized by the finding that ectopic expression of only a few transcription factors

© 2011 Elsevier Ltd. All rights reserved.

Corresponding author: Bernstein, E. (emily.bernstein@mssm.edu).

*These authors contributed equally and were placed in order of last names.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(TFs) can induce pluripotency [2, 3]. Such reprogrammed cells are referred to as induced pluripotent stem cells (iPSCs), and allow researchers vast opportunities to study human stem cell biology in an ethical fashion, numerous diseases using patient-derived iPSCs, and circumvents the ethical issues that can arise with somatic cell nuclear transfer (SCNT). Although the molecular mechanisms underlying ESC and SCR biology are beginning to unfold, further studies are indeed required to facilitate future advances in this exciting area.

In particular, it is becoming clear that, in addition to the transcriptional networks that govern i) the ESC state, ii) differentiation into particular lineages, and iii) SCR, there is a significant contribution from the epigenome. Mechanisms that regulate the epigenome include distinct enzymatic complexes that directly contribute to DNA and chromatin modification, effector proteins that bind to these modifications, chromatin remodeling, as well as global chromatin reorganization – all of which allow for dramatic changes to occur during cell fate transitions. Such chromatin dynamics are discussed in detail in this review, as well as the concept of the ‘epigenetic barrier’. In order for the nucleus of a somatic cell to be re-configured during reprogramming, a crucial barrier comprised of epigenetic modifications needs to be surmounted. In contrast to review articles focusing on the transcriptional networks and signaling pathways required for ESC maintenance, or the advancement of methods for iPSC-derivation [4, 5], here we focus on the epigenetic landscape of ESCs, their differentiated progeny, and SCR.

The ESC epigenetic landscape

Open Chromatin of ESCs

It is well established that the maintenance of ESC self-renewal requires an interconnected network of TFs, including Oct4, Sox2 and Nanog [4]. More recently, chromatin regulators have come into light for their roles in the maintenance ESC self-renewal and pluripotency (see below). ESCs possess multiple distinctive epigenetic features. ESC chromatin is ‘hyperdynamic’ and considered more ‘open’ than that of their differentiated progeny [6] (Figure 1). ESCs also have a highly active transcriptome and contain robust chromatin remodeling activities [7]. This hyperdynamic state of ESCs is thought to allow for efficient chromatin reorganization that takes place during lineage specification [8] (Figure 1). Here we discuss the roles played by chromatin regulators in the maintenance of this unique chromatin state.

Chromatin remodelers

ATP-dependent chromatin remodeling complexes regulate interactions between histone-octamers and the DNA-helix, thereby modulating DNA accessibility to TFs or other chromatin-associated factors [9]. For example, in mouse ESCs (mESCs), a unique SWI/SNF complex termed esBAF has been identified. This complex consists of the ATPase BRG1 (SMARCA4), and a unique set of regulatory subunits (BAFs) that are critical for its function in ESCs [10]. Brg1 maintains self-renewal by directly regulating the expression of Oct4, Sox2 and Nanog, and perturbation of BRG1 activity induces ESC differentiation [10]. Recent data from our research team suggests an additional role for SWI/SNF complexes. Apart from ‘fine-tuning’ self-renewal, BAF subunits are required for Nanog repression, heterochromatin formation, and chromatin compaction during differentiation [11]. This suggests context-dependent roles for these multi-subunit remodeling complexes.

Several subunits of the Mi-2/nucleosome remodeling and deacetylase (NuRD) complex, which possesses ATP-dependent nucleosome remodeling and histone deacetylase (HDAC) activities, play crucial roles in ESC self-renewal, pluripotency and embryogenesis [12, 13]. Homozygous deficiency of the non-catalytic subunit, Mbd3, allows mESCs to bypass leukemia inhibitory factor (LIF) dependency (a key signaling molecule required for the

mESC undifferentiated state (see ref. [5]), and impairs mESC differentiation [13]. A similar complex termed NODE (Nanog and Oct4 associated deacetylase), which shares subunits with NURD, has also been identified in mESCs [12]. It remains to be determined how these two NURD-like complexes are assembled, targeted to specific genomic regions, and alter chromatin structure to direct cell fate decisions.

In a recent RNAi screen of ~1000 chromatin factors, Tip60-p400 was identified as essential for mESC pluripotency [14]. Like NURD, the TIP60-p400 complex exhibits multiple functions, including histone variant exchange and histone acetyltransferase (HAT) activities. Depletion of Tip60, p400 or any other complex members triggers mESC differentiation [14]. Through an RNAi screening approach, CHD1 was identified as a factor required to maintain an 'open' chromatin conformation in mESCs. The chromodomain-helicase-DNA-binding (CHD) family of proteins is characterized by a chromodomain (CD), a SNF2-related helicase/ATPase and a DNA binding domain. Depletion of CHD1 induces heterochromatin formation, enhances propensity for neural differentiation and impedes effective SCR [15]. Using hESC-derived human neural crest-like cells (hNCLCs), another CHD member, CHD7, was found to be required for the formation of migratory-competent hNCLCs, through its interaction with the PBAF complex [16]. However, precise mechanistic understanding of how CHD members fulfill such roles in maintaining the undifferentiated state or lineage commitment remains to be determined.

Histone Modifying Complexes

Histone modifying enzymes covalently modify histone tail residues, and are commonly referred to as 'writers'. 'Readers' (or effectors) specifically recognize and bind such modifications and facilitate downstream chromatin events, which may include chromatin remodeling or chromatin compaction. Many 'writers' and 'readers' have been functionally characterized or implicated in the context of ESC self-renewal, early embryonic development and SCR (Table 1). To date, the Polycomb Group (PcG) proteins have received significant attention in this regard. PcG proteins are transcriptional repressors that function as multimeric complexes termed Polycomb Repressive Complex 1 (PRC1) or 2 (PRC2). PRC2 contains histone methyltransferase (HMT) activity for H3K27 and PRC1 contains a CD-containing Pc protein (known as CBXs in mammals) that binds to this modification (Table 1). Functional inactivation of selective PcG members results in early mouse embryonic lethality, as well as defects in mESC differentiation [17] (Table 1). This is due to the fact that, in both hESCs and mESCs, PcG proteins regulate the expression of developmentally associated TFs, such as the Hox, Sox, Pou, Pax, Fox and Tbx families, among others [17].

Recently, several studies investigated PRC2-interacting proteins in ESCs and identified Jarid2, a Jumonji C (JMJC) domain-containing protein, as a novel component [18]. Jarid2 is required for recruitment of PRC2 to its target loci and modulates PRC2 methyltransferase activity. Another study showed Polycomb-like protein 2 (PCL2) to interact with PRC2 and to be required for transcriptional modulation of self-renewal genes, as well as developmental regulators during early lineage commitment [19].

Intriguingly, very few complementary studies exist for Trithorax Group (TrxG) proteins in ESCs. The presence of bivalent domains in ESCs (characterized by co-occupancy of TrxG-mediated transcriptionally activating H3K4me3 and PcG-mediated repressive H3K27me3 marks) supports the notion that developmentally-regulated gene expression is tightly coordinated by these two antagonistic complexes [20–22]. We anticipate that further investigation of the roles played by TrxG proteins in ESCs will enhance the understanding of ESC identity, regulation of early embryonic development and SCR.

The irreversible silencing of Oct4 expression in somatic cell lineages is mediated by a multi-step process that involves inhibition of transcription, through methylation of both H3K9 and DNA. The HMT G9a is crucial for this process. G9a induces H3K9 dimethylation, and promotes *de novo* DNA methylation by DNMT3a/3b at the *Pou5f1* locus during ESC differentiation [13]. Interestingly, differentiation of mESCs induces the formation of large-scale (~2Mb) modified genomic regions termed Large Organized Chromatin K9 (LOCKs), which are also dependent on G9a activity [23]. Another H3K9 HMT, ESET (Setdb1), is critical for both early mouse development and maintenance of mESC self-renewal. ESET maintains repression of genes encoding developmental regulators and restricts extra-embryonic trophoblast lineage differentiation through its interaction with Oct4 [24–26]. Conversely, two H3K9 demethylases, Jmjd1a and Jmjd2c, are required for the maintenance of mESC self-renewal [27]. Both Jmjd1a and Jmjd2c gene promoters are directly occupied and transcriptionally regulated by Oct4, and in turn Jmjd1a/2c proteins regulate pluripotency by modulating the expression of key transcription factors Tcf1 and Nanog, respectively, by demethylating H3K9me at their promoters. These results highlight the importance of regulating H3K9me levels in ESCs, which might tip the balance between self-renewal and differentiation.

Histone Variants and Chaperones

A special feature of ESC chromatin is that developmentally regulated genes are silenced, but remain poised for activation upon lineage commitment, mediated in part by bivalent domains. Intriguingly, the histone variant H2A.Z is also enriched at transcription start sites of developmentally regulated genes [28]. Depletion of H2A.Z results in increased expression of such genes in mESCs, and ChIP-chip studies of H2A.Z show a strong overlap in genome-wide binding targets with PRC2 subunit Suz12. Another histone variant recently implicated in ESC biology is H3.3 [29]. Using ChIP-seq in mESCs, H3.3 was found to be enriched at both active and repressed genes, as well as at telomeres. This is somewhat unexpected since H3.3 has been primarily described as a mark of active chromatin [30]; however, it is becoming clear that we still have much to learn about histone variant biology. For example, distinct histone chaperone complexes regulate H3.3 incorporation into euchromatic (HIRA) and heterochromatic regions (ATRX/DAXX), indicating a high level of complexity in histone variant biology and the chromatin organization of ESCs [31].

Chromatin Dynamics of ESC Differentiation

Although ESCs maintain pluripotency and self renew, they can also differentiate down specific lineages if given the proper stimuli. Stem cells, whether embryonic or adult in origin, undergo drastic gene expression profile changes throughout the process of differentiation and such changes must be tightly regulated such that a cell adopts a specific lineage fate [32]. In order to achieve such specification, stem cells remodel and significantly alter their chromatin architecture.

One striking example is the X inactivation process in female mammals. This step-wise heterochromatin assembly phenomenon inactivates one of the two female X chromosomes for gene dosage compensation. X inactivation during mESC differentiation is driven predominantly by Xist-mediated RNA coating of the X chromosome, followed by repressive histone modifications H3K9 and H3K27 trimethylation, DNA methylation and incorporation of the histone variant macroH2A [33]. Intriguingly, female hESCs display a highly variable epigenetic state of the X chromosome, ranging from partial to complete X inactivation. However, these inactivated X chromosomes lack XIST expression in the undifferentiated state and do not reactivate XIST upon lineage commitment [33, 34]. However, the derivation of hESCs from human blastocysts under low, physiological oxygen concentrations has resulted in hESC lines containing two active X's, one of which becomes

randomly inactivated upon differentiation (akin to the process in mESCs) [35]. This suggests that the human blastocyst does indeed contain cells with two active X chromosomes. While X inactivation involves remodeling of an entire chromosome, global chromatin changes during differentiation have also been observed. For example, during early embryonic specification, a higher level of global chromatin compaction is observed *in vivo* in lineage-committed cells as compared to the pluripotent epiblast (cells that will give rise to the whole organism), through the use of electron spectroscopic imaging [36].

Although many epigenetic regulators might in fact be dispensable for the maintenance of the undifferentiated state, they are essential for the execution of cellular differentiation - PcG proteins being a prime example [37]. In general, ESCs lacking epigenetic regulators tend to exhibit defective differentiation phenotypes (described above), reflecting their role in determining and engaging particular lineage specific programs. The re-introduction of such regulators in ESCs restores differentiation ability, indicating that pluripotency has remained intact [37]. For example, the expression of Mbd3 in an Mbd3 null background enables ESCs to differentiate properly [13], and by expressing G9a in G9a-deficient cells, H3K9me2 levels are restored [38]. Therefore, it is becoming clear that understanding epigenetic regulation is critical to draw a full picture of how undifferentiated cells maintain their state and execute differentiation pathways. In fact, some cell lineages are still difficult to obtain with current differentiation protocols. However, it may be possible to overcome such barriers by modulation of yet unidentified chromatin factors. Thus far, the majority of protocols modulate signaling pathways and TFs; however, chromatin factors may be critical in directing cell fates. For example, the chromatin remodeling subunit Baf60c has been used to direct ectopic differentiation of mouse mesoderm into beating cardiomyocytes [39]. Transdifferentiation, the process whereby somatic cells can be directly converted from one cell type to another, may also be mediated by chromatin factors. To date, however, mainly TFs have been used for such reprogramming of cell fate [40].

Reversibility of the pluripotency program

In recent years, an increased interest in pluripotent stem cells has erupted due to their pluripotent and self-renewing capacities, and consequently, their use as models to study early development and disease [41]. As embryonic development is a unidirectional process, there is a progressive loss of differentiation potential. To explore the reversibility of this process, early work in the frog demonstrated that a differentiated nucleus could regain pluripotency by transfer into an enucleated oocyte (SCNT) [42]. In recent years, other techniques have proven to be useful for reprogramming, such as cell fusion of somatic cells with pluripotent cells or through the ectopic expression of TFs, giving rise to iPSCs. Initially, iPSCs were obtained from mouse fibroblasts through expression of TFs highly represented in ES cells (Oct4, Sox2, cMyc and Klf4) [2]. Similarly, human somatic cells have been converted into iPSCs using these four factors, or with different combinations of factors, including human OCT4, SOX2, LIN28 and NANOG [3]. More recently, by using other sources of somatic cells such as dermal papilla cells [43] or neural stem cells [44], iPSCs were derived through ectopic expression of Oct4 and Klf4, or solely with Oct4, respectively.

One of the key questions that arises from this phenomenon is how do these TFs act to induce pluripotency? It is well known that Oct4, Nanog and Sox2 are part of a crucial autoregulatory loop that maintains pluripotency in ESCs and that cMyc binds to genes not bound by Oct4, Sox2 and Klf4 [45]. Also, we ponder, how does this well-established transcriptional network converge with chromatin regulators to mediate SCR? Evidence suggests that the ESC transcriptional and epigenetic networks crosstalk at several levels. For example, cMyc seems to play an important role in release of transcriptional pausing of the

majority of active genes in ESCs (and possibly other proliferating cells) in concert with the elongation factor pTEFb [46]. Its role may be connected with chromatin reorganization, where the formation of nucleosome-depleted regions facilitates RNA pol II transcription [47]. However, cMyc is not essential for reprogramming, as it can be substituted by other factors such as chromatin regulators (see below). As another example, the chromatin structure of the extended Nanog locus (which also includes the pluripotent-associated genes Apobec, GDF3 and Dppa3) has been shown to be dependent on Oct4 [48]. Furthermore, Oct4-interactome studies have identified a plethora of novel Oct4-interacting partners; many of which are chromatin-associated proteins [49, 50]. This includes subunits of the NURD, SWI/SNF, lysine-specific demethylase 1 (LSD1) complexes [49, 50]; PRC1 and TRRAP/p400 complexes [50] as well as the INO80 chromatin-remodeling complex, FACT, histone chaperones, histone ubiquitination/E3 ubiquitin ligase complexes, major histones and helicases [49]. In short, these results suggest cooperativity between the reprogramming TFs and selected chromatin-associated molecules for the maintenance and induction of pluripotency.

Epigenetic memory

Although the induction of pluripotency through iPS clearly involves epigenetic reprogramming, and it has been suggested that the epigenome of iPSCs is similar to that of ESCs [51], more recent studies suggest that the genomic and epigenomic signatures are not fully reset [52–54]. A thorough molecular analysis of the expression profiles of iPSCs, comparing different reprogramming strategies and different passages in culture, indicates that the differences between ESCs and iPSCs are clearly acknowledgeable, but can be attenuated through passages in culture. These differences may be due to the source of the cells themselves or the technology used to reprogram, but it is intriguing that culturing iPSCs over time also affects the reprogramming state [52]. Recent evidence suggests that iPSCs retain an ‘epigenetic memory’ of the donor tissue they are derived from. Certain iPSCs may favor differentiation along lineages related to the donor cell, thereby restricting alternative cell fates [53, 54]. The mechanisms responsible for maintaining or removing such ‘epigenetic memory’ in iPSCs remain to be determined, but clearly involve DNA methylation [54].

Another example of differences between ESCs and iPSCs was found in some, but not all iPSC lines, that were not able to contribute to chimeric mice and support the development of entirely iPSC-derived animals. Such iPSC lines were found to have the Dlk1-Dio3 imprinted cluster (which includes several microRNAs and genes Gtl2, Ryan and Mirg) strongly repressed with a concomitant reduction of histone acetylation [55]. This aberrant epigenetic silencing could be overcome using a histone deacetylase (HDAC) inhibitor to reactivate the locus, thereby allowing these cells to give rise to embryos fully originated from iPSCs. The caveat here was that the pups derived from these cells were not viable, possibly due to some secondary effects of the treatment with the HDAC inhibitor valproic acid (VPA) [55].

It is likely that the genome wide comparative analyses performed thus far between iPSCs and ESCs may have missed key genomic regions. Repetitive elements of the genome (mostly in centromeric and telomeric regions) remain difficult to analyze with current technologies, and it is possible that some differences may exist in these unexplored regions. We also favor the view that histone variants play a role in epigenetic memory and regulate the ‘epigenetic barrier’ during SCR. Given the fact that most histone variants are incorporated into chromatin in a replication-independent manner (unlike the canonical histones), the possibility for fully differentiated cells to mark particular regions of the genome with unique histone variants may contribute to the epigenetic barrier during reprogramming [56].

Breaking the epigenetic barriers

In general, iPS is a very inefficient process. However, eventually all cells can complete the reprogramming process [57], demonstrating the stochastic nature of this process. The reprogramming process is slow and gradual, with several intermediate states [58]. Moreover, reactivation of endogenous ESC genes such as Oct4 (visualized in an Oct4-GFP reporter cell line) showed that even similar morphological iPS colonies start expressing Oct4 at different times [59]. This might be conferred in part by stochastic epigenetic events.

It appears that SCR involves massive reconfiguration of chromatin structure, from DNA methylation, to histone modifications, to nucleosome remodeling. These present themselves as 'epigenetic barriers' during SCR, as they are generally used as repressive mechanisms in somatic cells to prevent unwanted gene expression from other lineages. How these barriers are overcome is a central question, and remains a black box (Figure 1). However, emerging evidence suggests that epigenetic factors are at play [58, 59]. For example, the substitution of Myc with BAF components during reprogramming allows for Oct4 reactivation and full reprogramming of somatic cells [13]. The use of epigenetic inhibitors also demonstrates the importance in reprogramming the epigenome. For example, inhibition of DNA methylation with the DNA methyltransferase (DNMT) inhibitor 5-aza-cytidine (5-AZA) during reprogramming allows intermediate iPSCs to be fully reprogrammed [60]. Inhibition of histone deacetylation using the HDAC inhibitors valproic acid (VPA) and trichostatin A (TSA) also improves SCR efficiency [61] [13] (Table 1). In fact, treatment with TSA alone (without transduction of the reprogramming factors) is sufficient to upregulate ESC-specific genes [61]. The use of Parnate (tranylcypromine), an inhibitor of LSD1, also increases the reprogramming efficiency of mouse fibroblasts [13]. Finally, induction of histone H3K9 hypomethylation using a G9a methyltransferase chemical inhibitor BIX-01294, enhances the reprogramming of neural precursor cells and MEFs into iPSCs [62]. There is also evidence for an 'epigenetic barrier' when down regulating epigenetic factors in the context of SCR (Figure 1). For example, loss of CHD1 in mouse somatic cells [15], and PRDM14 (a transcriptional regulator with a SET domain of unknown function) in human cells [63], results in decreased reprogramming efficiency. Clearly, reprogramming is a very complex sequence of events that involves silencing of the somatic cell program, resetting of the self-renewing and pluripotency programs, and surmounting epigenetic barriers.

Concluding remarks

Chromatin dynamics are essential for ESC maintenance, cell lineage specification and reprogramming. Although much attention has recently focused on advancing methods for i) directing lineage specification of ESCs [41], ii) technologies to enhance reprogramming efficiencies and safe delivery of exogenous TFs in various types of somatic cells [3], iii) replacement of the aforementioned TFs with small molecule compounds [13, 60], and iv) comparing the genomic and epigenomic features of ESCs and iPSCs [51, 64, 65]; significantly less attention has been focused on the epigenetic factors or barriers that drive differentiation or must be surmounted during reprogramming, respectively. Advances in the knowledge of chromatin regulation during ESC differentiation and reprogramming will greatly enhance our ability to design rationally guided stem cell therapies (by either directing specific cell lineages or reprogramming them) as well as potential novel therapeutic approaches.

Acknowledgments

The authors thank Hsan-Au Wu for critically reading this manuscript. We apologize to those whose work could not be cited due to space limitations. A NYSTEM IDEA Award C024285 to E.B., NYSTEM Award C024410 and National Institutes of Health GM078465 to I.R.L., support this work.

Glossary

Epigenome	The landscape of epigenetic modifications that occurs across the genome, encompassing the covalent and non-covalent modifications of DNA and histone proteins, which in turn, influences the overall chromatin structure (i.e. posttranslational modifications of histones, incorporation of histone variant proteins, and DNA methylation)
Epigenetic memory	Epigenetic modifications that can be retained upon reprogramming or cell divisions from a donor cell. The ability of reprogrammed cells to give rise to fully developed organisms varies significantly, depending on the cell source. These variations have been attributed to differences in cell-type specific epigenetic information
Undifferentiated state	The undifferentiated state or pluripotent state of ESCs is defined as self-renewing with the ability to form all three lineages of the body (endoderm, mesoderm and ectoderm). Differentiated cells are therefore cells already committed to a particular lineage

References

1. Martin G. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *PNAS*. 1981; 78(12):7634–7638. [PubMed: 6950406]
2. Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell*. 2006; 126(4):663–676. [PubMed: 16904174]
3. Yamanaka S. A fresh look at iPS cells. *Cell*. 2009; 137(1):13–7. [PubMed: 19345179]
4. MacArthur BD, Ma'ayan A, Lemischka IR. Systems biology of stem cell fate and cellular reprogramming. *Nat Rev Mol Cell Biol*. 2009; 10(10):672–81. [PubMed: 19738627]
5. Li W, Ding S. Small molecules that modulate embryonic stem cell fate and somatic cell reprogramming. *Trends Pharmacol Sci*. 2010; 31(1):36–45. [PubMed: 19896224]
6. Meshorer E, et al. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Developmental Cell*. 2006; 10(1):105–16. [PubMed: 16399082]
7. Efroni S, et al. Global transcription in pluripotent embryonic stem cells. *Cell Stem Cell*. 2008; 2(5): 437–47. [PubMed: 18462694]
8. Niwa H. Open conformation chromatin and pluripotency. *Genes & Development*. 2007; 21(21): 2671–6. [PubMed: 17974911]
9. Ho L, Crabtree GR. Chromatin remodelling during development. *Nature*. 2010; 463(7280):474–84. [PubMed: 20110991]
10. Ho L, et al. An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. *Proc Natl Acad Sci USA*. 2009
11. Schaniel C, et al. Smarcc1/Baf155 couples self-renewal gene repression with changes in chromatin structure in mouse embryonic stem cells. *Stem Cells*. 2009; 27(12):2979–91. [PubMed: 19785031]
12. Liang J, et al. Nanog and Oct4 associate with unique transcriptional repression complexes in embryonic stem cells. *Nat Cell Biol*. 2008; 10(6):731–9. [PubMed: 18454139]
13. .
14. Fazio TG, Huff JT, Panning B. An RNAi Screen of Chromatin Proteins Identifies Tip60-p400 as a Regulator of Embryonic Stem Cell Identity. *Cell*. 2008; 134(1):162–74. [PubMed: 18614019]
15. Gaspar-Maia A, et al. Chd1 regulates open chromatin and pluripotency of embryonic stem cells. *Nature*. 2009; 460(7257):863–8. [PubMed: 19587682]
16. Bajpai R, et al. CHD7 cooperates with PBAF to control multipotent neural crest formation. *Nature*. 2010; 463(7283):958–62. [PubMed: 20130577]
17. Surface LE, Thornton SR, Boyer LA. Polycomb group proteins set the stage for early lineage commitment. *Cell Stem Cell*. 2010; 7(3):288–98. [PubMed: 20804966]
18. Landeira D, Fisher AG. Inactive yet indispensable: the tale of Jarid2. *Trends Cell Biol*. 2010

19. Walker E, et al. Polycomb-like 2 associates with PRC2 and regulates transcriptional networks during mouse embryonic stem cell self-renewal and differentiation. *Cell Stem Cell*. 2010; 6(2): 153–66. [PubMed: 20144788]
20. Zhao X, et al. Whole-Genome Mapping of Histone H3 Lys4 and 27 Trimethylations Reveals Distinct Genomic Compartments in Human Embryonic Stem Cells. *Cell Stem Cell*. 2007; 1(3): 286–298. [PubMed: 18371363]
21. Bernstein BE, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*. 2006; 125(2):315–26. [PubMed: 16630819]
22. Ringrose L, Paro R. Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development*. 2007; 134(2):223–32. [PubMed: 17185323]
23. Wen B, et al. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat Genet*. 2009
24. Bilodeau S, et al. SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state. *Genes Dev*. 2009; 23(21):2484–9. [PubMed: 19884255]
25. Yeap LS, Hayashi K, Surani MA. ERG-associated protein with SET domain (ESET)-Oct4 interaction regulates pluripotency and represses the trophectoderm lineage. *Epigenetics Chromatin*. 2009; 2(1):12. [PubMed: 19811652]
26. Yuan P, et al. Eset partners with Oct4 to restrict extraembryonic trophoblast lineage potential in embryonic stem cells. *Genes Dev*. 2009; 23(21):2507–20. [PubMed: 19884257]
27. Loh YH, et al. Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. *Genes & Development*. 2007; 21(20):2545–57. [PubMed: 17938240]
28. Creighton MP, et al. H2AZ is enriched at polycomb complex target genes in ES cells and is necessary for lineage commitment. *Cell*. 2008; 135(4):649–61. [PubMed: 18992931]
29. Goldberg AD, et al. Distinct Factors Control Histone Variant H3.3 Localization at Specific Genomic Regions. *Cell*. 2010; 140(5):678–691. [PubMed: 20211137]
30. Ahmad K, Henikoff S. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Molecular Cell*. 2002; 9(6):1191–200. [PubMed: 12086617]
31. Lewis PW, et al. Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. *Proc Natl Acad Sci U S A*. 2010; 107(32):14075–80. [PubMed: 20651253]
32. Rossant J. Stem cells and early lineage development. *Cell*. 2008; 132(4):527–31. [PubMed: 18295568]
33. Payer B, Lee JT. X chromosome dosage compensation: how mammals keep the balance. *Annu Rev Genet*. 2008; 42:733–72. [PubMed: 18729722]
34. Bernstein E, et al. A phosphorylated subpopulation of the histone variant macroH2A1 is excluded from the inactive X chromosome and enriched during mitosis. *Proc Natl Acad Sci USA*. 2008; 105(5):1533–8. [PubMed: 18227505]
35. Lengner CJ, et al. Derivation of pre-X inactivation human embryonic stem cells under physiological oxygen concentrations. *Cell*. 2010; 141(5):872–83. [PubMed: 20471072]
36. Ahmed K, et al. Global chromatin architecture reflects pluripotency and lineage commitment in the early mouse embryo. *PLoS ONE*. 2010; 5(5):e10531. [PubMed: 20479880]
37. Niwa H. How is pluripotency determined and maintained? *Development*. 2007; 134(4):635–46. [PubMed: 17215298]
38. Tachibana M, et al. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes & Development*. 2002; 16(14):1779–91. [PubMed: 12130538]
39. Takeuchi JK, Bruneau BG. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. *Nature*. 2009; 459(7247):708–11. [PubMed: 19396158]
40. Jopling C, Boue S, Izpisua Belmonte JC. Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. *Nat Rev Mol Cell Biol*. 2011; 12(2):79–89. [PubMed: 21252997]
41. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell*. 2008; 132(4):661–80. [PubMed: 18295582]

42. Gurdon JB, Melton DA. Nuclear reprogramming in cells. *Science*. 2008; 322(5909):1811–5. [PubMed: 19095934]
43. Tsai S-Y, et al. Oct4 and Klf4 Reprogram Dermal Papilla Cells into Induced Pluripotent Stem Cells. *Stem Cells*. 2009 p. N/A-N/A.
44. Kim JB, et al. Oct4-induced pluripotency in adult neural stem cells. *Cell*. 2009; 136(3):411–9. [PubMed: 19203577]
45. Kim J, et al. An extended transcriptional network for pluripotency of embryonic stem cells. *Cell*. 2008; 132(6):1049–61. [PubMed: 18358816]
46. Rahl PB, et al. c-Myc regulates transcriptional pause release. *Cell*. 2010; 141(3):432–45. [PubMed: 20434984]
47. Knoepfler PS. Why myc? An unexpected ingredient in the stem cell cocktail. *Cell Stem Cell*. 2008; 2(1):18–21. [PubMed: 18371417]
48. Levasseur DN, et al. Oct4 dependence of chromatin structure within the extended Nanog locus in ES cells. *Genes & Development*. 2008; 22(5):575–80. [PubMed: 18283123]
49. Pardo M, et al. An expanded Oct4 interaction network: implications for stem cell biology, development, and disease. *Cell Stem Cell*. 2010; 6(4):382–95. [PubMed: 20362542]
50. van den Berg DLC, et al. An Oct4-centered protein interaction network in embryonic stem cells. *Cell Stem Cell*. 2010; 6(4):369–81. [PubMed: 20362541]
51. Maherali N, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*. 2007; 1(1):55–70. [PubMed: 18371336]
52. Chin MH, et al. Molecular analyses of human induced pluripotent stem cells and embryonic stem cells. *Cell Stem Cell*. 2010; 7(2):263–9. [PubMed: 20682452]
53. Polo JM, et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol*. 2010; 28(8):848–55. [PubMed: 20644536]
54. Kim K, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010
55. Stadtfeld M, et al. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature*. 2010; 465(7295):175–81. [PubMed: 20418860]
56. Ng RK, Gurdon JB. Epigenetic memory of an active gene state depends on histone H3.3 incorporation into chromatin in the absence of transcription. *Nat Cell Biol*. 2008; 10(1):102–9. [PubMed: 18066050]
57. Hanna J, et al. Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature*. 2009; 462(7273):595–601. [PubMed: 19898493]
58. Sridharan R, et al. Role of the murine reprogramming factors in the induction of pluripotency. *Cell*. 2009; 136(2):364–77. [PubMed: 19167336]
59. Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol*. 2007; 25(10):1177–81. [PubMed: 17724450]
60. Mikkelsen TS, et al. Dissecting direct reprogramming through integrative genomic analysis. *Nature*. 2008; 454(7200):49–55. [PubMed: 18509334]
61. Huangfu D, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol*. 2008; 26(7):795–7. [PubMed: 18568017]
62. Shi Y, et al. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. *Cell Stem Cell*. 2008; 2(6):525–8. [PubMed: 18522845]
63. Chia N-Y, et al. A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. *Nature*. 2010
64. Marchetto MCN, et al. Transcriptional signature and memory retention of human-induced pluripotent stem cells. *PLoS ONE*. 2009; 4(9):e7076. [PubMed: 19763270]
65. Chin MH, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*. 2009; 5(1):111–23. [PubMed: 19570518]

Figure 1. Breaking the epigenetic barrier

ESCs have a transcriptionally permissive chromatin structure, allowing pluripotency genes to be expressed (marked by H3K4me3, histone acetylation, RNA polymerase II, and devoid of promoter DNA methylation), whereas lineage-specific genes X and Y are repressed and marked by bivalent domains (H3K4me3 and H3K27me3) to allow for activation upon differentiation signals. During ESC differentiation, re-organization of the chromatin structure occurs and becomes increasingly heterochromatic (marked by H3K9me3 and its reader HP1, as well as by promoter DNA methylation), in part to silence ESC-specific genes and other lineage specific genes (i.e. the Y lineage). Upon reprogramming, ESC-specific genes must be re-activated and this poses an obstacle for the cell - the so-called 'epigenetic barrier' (black box). In order to overcome this barrier, the epigenome must be remodeled, and successful methods to achieve this include addition of the DNMT inhibitor, 5-Azacytidine and HDAC inhibitors such as TSA and VPA. In addition, chromatin remodelers such as BRG1 help to break the epigenetic barrier. Although CHD1 is necessary for reprogramming, its expression has not yet been shown to be sufficient to increase efficiency of the process. Histone H3 trimethylation marks are depicted by circles; histone acetylation by squares; DNA methylation by small filled circles and unmethylated DNA by small open circles.

Table 1

Histone modifying complexes and their roles in ESC maintenance, iPSC derivation and mouse development.

Complex ¹	Subunits in M.mus ² (UniProt ID)	PTM ³	ESC phenotype ⁴ (PMID)	OSN bound ⁵	ON interacting ⁶	ChIP-chip/seq datasets ⁷	mRNA in differentiation ⁸	mRNA in iPS formation ⁹	Mouse KO phenotype ¹⁰ (PMID)	Drug targeting ¹¹ (PMID)
PRC1	Rnf2 ¹ (Q9CQJ4)	H2AK119ub1	Rnwl (18339675)	-	O	16625203	↔	↑	emb (12589020)	PRT4165(19956605)
	Ring1* (O35730)		Rnwl (18339675)	-	-		nd	nd	post (11060235)	
	Bmi1* (Q2LCS8)			OSN	-		↑	↓	post (7926765)	
	Pcgf2(P23798)			O	-		↓	nd	post (8625838)	
	Cbx2 (P30658)			-	-		↑	nd	emb (9043087)	
	Cbx4 (O55187)			-	-		↑	↓		
	Cbx6 (Q9DBY5)		Rnwl (19884255)	-	-		↔	↔		
	Cbx7 (Q8VDS3)		Rnwl (19884255)	OSN	-		↓	↑		
	Cbx8 (Q9QXV1)		Rnwl (19884255)	OS	-		nd	↔		
	Phc1 (Q64028)			OSN	O	16625203	↓	↑	emb (9367423)	
PRC2	Phc2 (Q9QWH1)			-	-		↑	nd	emb (16024804)	
	Phc3 (Q8CHP6)			-	-		nd	↓		
	Ezh1 ¹ (P70351)	H3K27me2/3	Diffn (19026780)	OSN	-	19026780	↔	↑		DZNep(17437993)
	Ezh2 ¹ (Q61188)	H3K27me2/3	Diffn (19026780)	OSN	-	20064375	↓	↑	emb (11390661)	
	Eed* (Q921E6)		Diffn (18403752)	OSN	-	16625203	↓	↑	emb (7768172)	
	Suz12* (Q80U70)		Diffn (17339329)	-	-	20064375	↔	↑	emb (15385962)	
	Rbbp4(Q60972)			-	-		↓	nd		
	Rbbp7 (Q60973)			OSN	ON		↔	nd		
	MLL ¹ (P55200)	H3K4me1/2/3	Rnwl (19884255)	O	-		↑	nd	emb (7477409)	DZNep(19509260)
	MLL2 ¹ (O08550)	H3K4me1/2/3		-	-		↔	nd	emb (16540515)	
Set/MLL	MLL3 ¹ (Q8BRH4)	H3K4me1/2/3		N	-		↑	nd		
	Setd1b ¹ (Q8CFT2)	H3K4me1/2/3		OSN	-		↓	nd		
	Setd7 ¹ (Q8VHL1)	H3K4me1/2/3	Rnwl (19884255)	O	-		↑	↓		
	Wdr5* (A2AKB1)			N	ON		↓	↑		Ac-ARA-NH(2) (20575550)
	Ash2L* (Q91X20)			OS	-		↓	↑	emb (20463296)	
	Rbbp5* (Q8BX09)			OSN	-		↓	↑		

Complex ¹	Subunits in <i>M.mus</i> ² (UniProt ID)	PTM ³	ESC phenotype ⁴ (PMID)	OSN bound ⁵	ON interacting ⁶	ChIP-chip/seq datasets ⁷	mRNA in differentiation ⁸	mRNA in iPS formation ⁹	Mouse KO phenotype ¹⁰ (PMID)	Drug targeting ¹¹ (PMID)
	HCFC1(Q61191)			-	O	20581084	↓	nd		
	DPY30(Q99LT0)			-	-		↓	↑		
Ese1/mAM	Setd1 ¹ (O88974)	H3K9me3	Rnw1(19884257)	-	O	19884255	↓	↑	emb(14993285)	
	Atf7ip(Q7TT18)			O	-		↔	↑		
G9a/GLP	Ehmt2 ¹ (Q9Z148)	H3K9me3	Diffh(16415856)	SN	-		↓	nd	emb(12130538)	BIX01294(189839700)
	Ehmt1(A2AIS3)		Rnw1(19339689)	OSN	-		↔	↔	emb(15774718)	
	Wiz(O88286)			-	-		↔	nd		
Suv(var)	Suv39H1 ¹ (O54864)	H3K9me3		-	-		↑	↔	post(11701123)	Chaetocin(16408017)
	Suv39H2 ¹ (Q9EQQ0)	H3K9me3	Rnw1(19884255)	-	-		↔	↑	post(11701123)	
Suv(var)	Suv420H1 ¹ (Q3U8K7)	H4K20me3		-	-		↓	nd		DZNep(17437993)
	Suv420H2 ¹ (Q6Q783)	H4K20me3		OS	-		↑	↑		
PR-Set7	Setd8 ¹ (Q2YDW7)	H4K20me1	Rnw1(18614019)	-	-		↔	↔	emb(19223465)	
Set2	Setd2 ¹ (Q6PCY9)	H3K36me3		-	-		↓	nd	emb(20133625)	
	Nsd1(O88491)			-	-		↔	nd	emb(12805229)	
Dot1	Dot1L ¹ (Q6XZL8)	H3K79me2/3	Diffh(19544450)	-	-		↔	nd	emby(18787701)	

¹ Generally accepted nomenclature of *H. sapiens*/*M. musculus* HMT-related complexes/enzymes.

² Subunits of named complexes. Official *M. musculus* gene name from Entrez-Gene with *M. musculus* protein ID from UniProtKB. [¶] indicates catalytic subunit. * indicates known core subunit required for enzymatic activity of complex. Interacting proteins that are not directly part of the complex (e.g. co-repressors/activators) are not included.

³ Post Translational Modification mediated by complex subunit. “1/2/3” indicates mono-, di-, or tri-methylation.

⁴ Phenotype described in ESCs. “Rnw1” indicates required for self-renewal maintenance in undifferentiated conditions. “Diffh” indicates required for proper differentiation. (PMID) indicates Pubmed ID of representative study.

⁵ Oct4 (O), Sox2 (S), Nanog (N) occupy the genomic locus of gene encoding the subunit (queried from Marson *et. al.* Cell 2009, TableS2) [66]. (-) indicates not bound.

⁶ Subunit interacts with protein of Oct4 (O) or Nanog (N) as queried from [(O) PMID-20362541/17093407/19811652] and [(N) PMID-17093407/18454139]. (-) indicates not interacting.

⁷ Pubmed ID from representative study of genome-wide ChIP-chip or ChIP-seq performed in ESCs for representative subunit.

⁸ Linear increase (↑) or decrease (↓) in mRNA expression during 14-day V6.5-ESC differentiation queried from GEO database (GDS2671 & GDS2672). (↔) indicates lack of a linear change. (nd) indicates absent call in microarray. Linear increase (↑) or decrease (↓) in mRNA expression during 21-day iPS formation from MEFs to iPSCs (queried from Samavarchi-Tehrani *et. al.* Cell Stem Cell 2010 TableS2) [67].

⁹ (↔) indicates lack of a linear change. (nd) indicates absent from dataset.

¹⁰ Mouse knockout phenotype of subunit. “Post” indicates post-natal defects; “emb” indicates embryonic defects. (PMID) indicates Pubmed ID of representative study.

¹¹ Chemical or peptide inhibitors targeting HMTs; might not be specific toward a single subunit. In grey are inhibitors not employed on ESCs or for iPSC derivation. (PMID) indicates Pubmed ID of representative study.