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Parallel gateways to pluripotency: open chromatin in stem cells and development

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Open chromatin is a hallmark of pluripotent stem cells, but the underlying molecular mechanisms are only beginning to be unraveled. In this review we highlight recent studies that employ embryonic stem cells and induced pluripotent stem cells to investigate the regulation of open chromatin and its role in the maintenance and acquisition of pluripotency *in vitro*. We suggest that findings from *in vitro* studies using pluripotent stem cells are predictive of *in vivo* processes of epigenetic regulation of pluripotency, specifically in the development of the zygote and primordial germ cells. The combination of *in vitro* and *in vivo* approaches is expected to provide a comprehensive understanding of the epigenetic regulation of pluripotency and reprograming.

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Introduction

Pluripotent stem cells have a limitless capacity for self-renewal and the unique potential to differentiate into all cell types. With the advent of techniques to reprogram somatic cells into pluripotent stem cells, there is an increased interest in understanding the mechanisms that underlie the maintenance and acquisition of pluripotency. Such understanding may provide important new insights into the regulation of embryonic development, and contribute to the generation of patient-specific pluripotent stem cells for disease modeling and cell replacement therapies.

While transcriptional differences between somatic cells and pluripotent stem cells are well established, there is increasing evidence supporting the critical role that chromatin accessibility plays in pluripotent stem cells. In this review, we highlight recent advancements in our understanding of how open chromatin regulates the maintenance and acquisition of pluripotency. We first describe epigenetic remodelers that regulate open chromatin *in vitro* in pluripotent embryonic stem (ES) cells and reprogramed induced pluripotent stem (iPS) cells. The large number of ES and iPS cells that can be grown *in vitro* has facilitated the dissection of epigenetic regulation of pluripotency in these cells. We then discuss the potential significance of these recent findings *in vivo*. We propose that epigenetic mechanisms used to maintain and acquire pluripotency *in vitro* operate *in vivo* in the acquisition of totipotency in the nascent zygote and maintenance of pluripotency in germ cells. The integration of studies *in vitro* and *in vivo* should thus significantly augment our global understanding of the epigenetic regulation of pluripotency and embryonic development.

ES cell cultures may reflect distinct *in vivo* epigenetic states

ES cells are pluripotent stem cells derived from the inner cell mass of the blastocyst before implantation, and they serve as an excellent *in vitro* model for probing the molecular mechanisms that govern cell fate decisions during early development. Recent data indicate that ES cells are not a homogeneous cell population as previously thought, but rather oscillate between different cell states that may have parallels *in vivo* [1–4,5••]. Mouse ES cell cultures contain significant heterogeneity: the core pluripotency gene Nanog [1] and stem cell markers Rex1 [2], Pecam1 [3], SSEA1 [3,4] and Stella [5••] have all been shown to exhibit a heterogeneous expression pattern, where ES cells are in flux between high and low expression of these genes. The variable phenotype correlates with *in vivo* expression patterns and appears to represent two distinct yet reversible embryonic stages: one that reflects an inner cell mass-like state, and another that is closer to an epiblast-like state [2,4,5••].

Strikingly, populations enriched for pluripotency markers SSEA1 or Stella are able to restore the original ratio of mixed populations [3,5••]. Stella expression levels correlate with the presence of activating histone marks H3K9ac and H3K4me3 at the Stella gene locus. Interestingly, the

Stella+ subpopulation is lost when ES cells are cultured in the absence of embryonic fibroblast feeder cells, and addition of the histone deacetylase inhibitor trichostatin A, which promotes active transcription, restores Stella expression in feeder-free conditions [5••]. Taken together, the data available suggest that extracellular signaling within ES cell cultures, and potentially *in vivo*, regulates gene expression and differentiation through epigenetic changes. Further evidence comes from a recent study demonstrating that ES cell-like cultures containing a Stella+ subpopulation can be derived directly from epiblast tissue or epiblast stem cells after prolonged culture with LIF-fetal calf serum on mouse embryonic fibroblast feeders [6]. This transformation is accompanied by DNA demethylation at the Stella and Rex gene loci, further supporting an epigenetic switch between the epiblast and inner cell mass-like states.

Direct evidence of epigenetic regulation of cell fate comes from studies of two closely related DNA-binding transcriptional regulators that are involved in higher order chromatin organization, Satb1 [7] and Satb2 [8]. The two Satb proteins appear to regulate ES cell self-renewal in an antagonistic manner: while both factors can bind to the Nanog promoter, Satb1 acts to repress Nanog transcription, and Satb2 appears to activate it. Therefore, the balance of Satb1 and Satb2 may underlie the heterogeneity of Nanog expression in ES cells [9]. However, it remains to be seen if extracellular signals lie upstream of these factors. It will be interesting in future studies to determine how LIF and other extracellular signals interact with epigenetic regulators to control pluripotency in ES cells and during development.

New epigenetic regulators of pluripotency

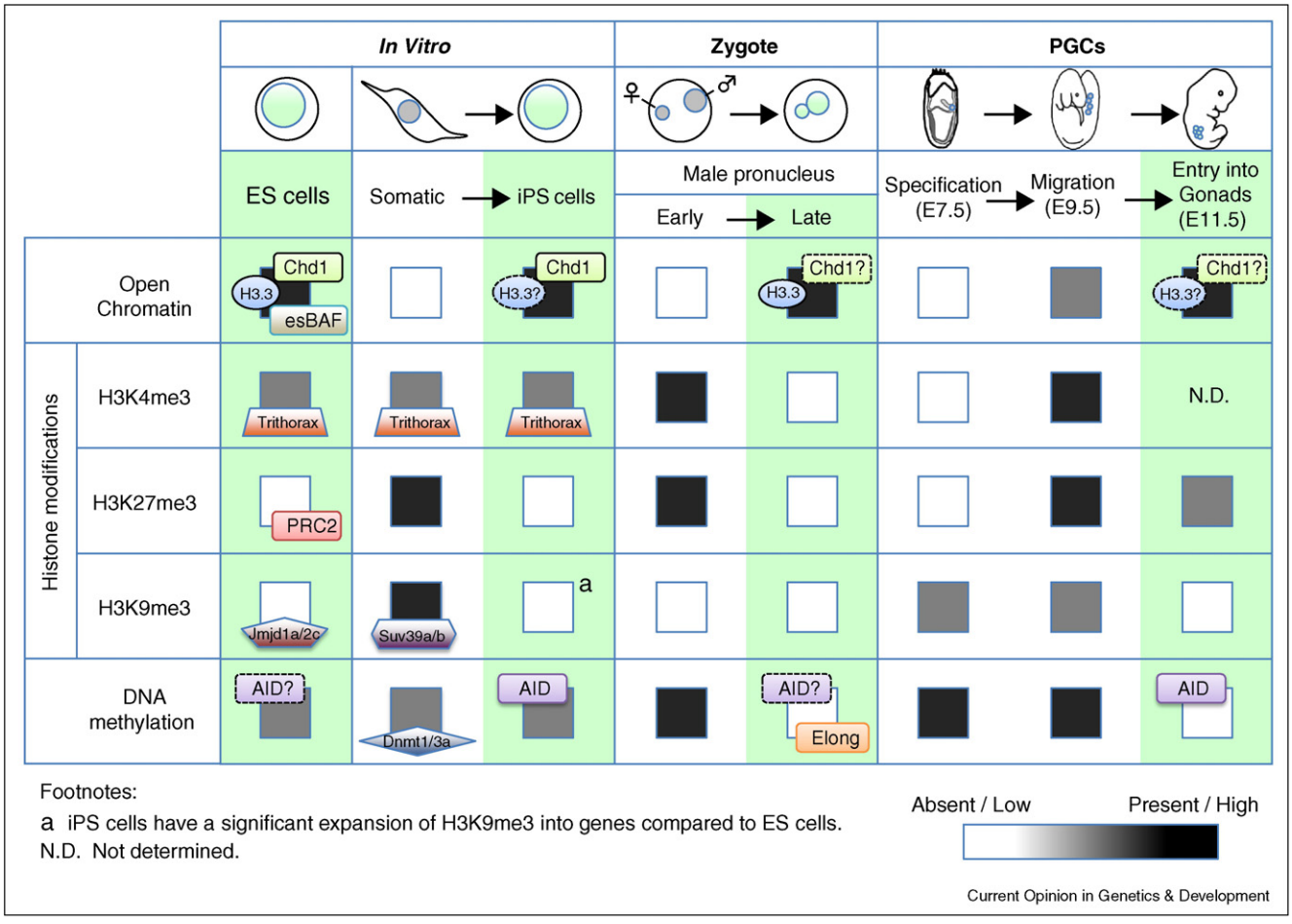
Pluripotent stem cells maintain a globally open chromatin state [10,11], possibly so that genes are readily available for activation during tissue specification [10]. ES cells have low levels of dense, compacted chromatin (heterochromatin) and the ES cell genome is transcriptionally hyperactive, with widespread transcription in both coding and noncoding regions, including sporadic low-level expression of tissue specific genes [10,12]. In addition, a recent study showed that the distribution of repressive marks H3K9me3 and H3K27me3 are significantly expanded in somatic cells relative to pluripotent stem cells [13•]. In agreement with these observations, chromatin remodeling factors are over-represented in the ES cell transcriptome, and RNAi knockdown of several chromatin remodelers like Chd1 and Brg1 has been shown to severely impact ES cell proliferation and differentiation potential [10,14••].

Chd1 is a chromatin remodeler associated with active transcription that contains a helicase domain, a DNA-binding domain and a pair of chromodomains that binds selectively to the euchromatin mark H3K4me2/3 [14••,15,16] (reviewed in [17]). Chd1 is required to maintain the open chromatin state of pluripotent mouse ES cells (Figure 1). Chd1-deficient ES cells show an increased number of heterochromatin foci and a pluripotency defect characterized by a high propensity for neural differentiation and an absence of primitive endoderm [14••]. The molecular mechanism by which Chd1 regulates open chromatin of ES cells remains unknown. Chromatin immunoprecipitation using promoter tiling arrays shows that Chd1 binding overlaps with markers of transcription, including RNAPolII and H3K4me3 [14••]. Interestingly, this distribution at gene promoters is similar to that of histone variant H3.3 [18]. H3.3 is correlated with sites of active transcription in many species [19–21] and appears to maintain open chromatin by inhibiting histone H1 binding to the nucleosome [22] (reviewed in [23]). The incorporation of H3.3 in ES cells is complex and includes the promoters of active and repressed genes, gene bodies only in active genes, transcriptional factor binding sites and telomeres [18]. Evidence from *Drosophila* suggests that Chd1 is required for H3.3 incorporation into chromatin (see below) [24]. It will therefore be of interest to characterize the genomic distribution of Chd1 binding in ES cells beyond gene promoters, determine which aspects of H3.3 incorporation, if any, are dependent on Chd1, and test whether H3.3 mediates the pluripotency defects in Chd1-deficient ES cells.

The BAF (Brg/Brahma-associated factors) complex is a chromatin remodeler of the SWI/SNF family that has been shown to regulate pluripotency [25••]. ES cells express a distinctive BAF complex (esBAF) defined by the presence of Brg1 (Brahma-related gene 1), BAF155, and BAF60A, and the absence of Brahma, BAF170, and BAF60C. Using genome-wide ChIP-seq technology, Brg1 was shown to colocalize extensively with the pluripotency transcription factors Oct4, Sox2, and Nanog, thereby suggesting a pluripotency-specific role for esBAF [26••]. In addition, Brg1 does not share many targets with the polycomb repressive complex PRC2, suggesting that esBAF is an activator of transcription.

Polycomb repressive complexes (PRCs) coordinate the transcriptional repression of lineage-specific developmental genes in ES cells in multiple ways, including mediating H3K27 dimethylations/trimethylations and H2A ubiquitination [27–29]. Disruption of either PRC1 or PRC2 results in early embryonic lethality *in vivo* [30–32]. This observation is mirrored *in vitro* by the propensity of PRC1-deficient or PRC2-deficient ES cells to differentiate [27,33]. Cell survival is greatly reduced upon initiation of differentiation in PRC-deficient ES cells, possibly because of activation of endogenous retroviruses [33]. Novel components of the PRC2 complex have recently been shown to be enriched in undifferentiated ES cells: Jarid2 was identified as a regulatory component

Figure 1



Potential parallels in epigenetic regulation of pluripotency in stem cells *in vitro* and the germline *in vivo*. ES and iPS cells are useful *in vitro* models to study the regulation of open chromatin and associated epigenetic marks in pluripotent cells. Several epigenetic regulators have been shown to be important for the establishment and maintenance of the open chromatin state of pluripotent stem cells, although the picture is far from complete. Much less is known about the epigenetic regulators that operate *in vivo* in epigenetic reprograming in the early zygote and PGCs. Note that the column 'zygote' refers to chromatin decondensation of the male pronucleus after fertilization. Recent evidence suggests that Chd1 and AID are essential chromatin regulators of pluripotency both *in vitro* and *in vivo*. Future studies may reveal the potential molecular parallels, as well as the differences, in epigenetic regulation between the various cell types depicted. Shaded columns represent cell types and states associated with open chromatin. For simplicity, several chromatin factors known to have roles in each specific cell type are not shown. See text for details.

that modulates PRC2 localization and activity [34••,35••], and Pcl2 was described as another component required for proper regulation of both pluripotency and lineage-specific genes in ES cells [36].

Finally, DNA methylation is another epigenetic mechanism by which ES cells may regulate gene expression. Recent studies challenge the classical view that ES cells have reduced global DNA methylation, but rather reveal that they use ES cell-specific non-CpG methylation in addition to the canonical CpG methylation [37,38•]. While DNA methylation is generally associated with transcriptional silencing, the functional significance of this alternative type of DNA methylation in ES cells remains to be determined. It should also be noted that a marker of active transcription, H3K36me3, is highly correlated with the presence of DNA methylation within gene bodies, suggesting a role for DNA methylation beyond transcriptional repression [13•,39]. Future investigations will be necessary to explore the functional significance of this alternative regulatory node.

In sum, recent findings indicate that the open, accessible chromatin state of ES cells is actively maintained by chromatin remodelers such as Chd1, and that PRCs and DNA methylation are involved in repression of developmental genes until differentiation is triggered. Therefore, a complex, dynamic balance is at play in the epigenetic regulation of ES cell pluripotency. How this balance may be re-established during reprograming

of somatic cells to pluripotency is the focus of the next section.

Reacquiring pluripotency *in vitro*: epigenetics is key

Cellular reprograming by the ectopic expression of defined transcription factors offers a reliable, albeit inefficient, method of obtaining iPS cells from somatic cells [40]. Although detailed analyses comparing mouse and human ES and iPS cells have uncovered subtle differences in gene expression patterns [41], iPS cells have consistently been found to reactivate pluripotency-related genes as well as re-establish an ES cell-like open chromatin state via global DNA demethylation and H3K4 and H3K27 methylation changes [41,42•,43]. Therefore, it is expected that global chromatin opening and DNA demethylation will be important components of reprograming (Figure 1).

In order to gain insight into the epigenetic changes that may underlie reprograming, several groups have compared stable partially reprogramed cell lines, which have not turned on the endogenous Oct4 gene, to true iPS cells that have activated Oct4 [42•,44,45]. These partially reprogramed cells exhibit DNA hypermethylation and a failure to remethylate H3K4 relative to ES and true iPS cells [42•], supporting the notion that proper epigenetic remodeling may be required for successful reprograming to pluripotency. Direct manipulation of the chromatin of these partially reprogramed cells and of somatic cells during reprograming have proved to be effective at enhancing reprograming efficiency. DNA methyltransferase inhibitor 5-azacytidine (5-azaC) induces partially reprogramed cells to undergo a rapid and stable transition to a fully reprogramed state [42•], while 5-azaC and histone deacetylase inhibitors like valproic acid increase the efficiency of iPS cell generation up to 40-fold [46]. These observations parallel earlier ones made using somatic cell nuclear transfer (SCNT), which established DNA demethylation of somatic cell nuclei as a necessary step to successful reprograming in mouse and *Xenopus* [47,48].

In addition, targeted downregulation by RNAi has enabled the identification of specific molecular complexes involved in the reprograming process. Downregulation of Chd1, a regulator of open chromatin and pluripotency of ES cells (see above), strongly inhibits generation of iPS cells [14••]. It will be of interest to determine whether H3.3 incorporation or other functions associated with Chd1 underlie its role in reprograming. Recently, activation-induced cytidine deaminase (AID), which is involved in DNA demethylation [49], has been implicated in reprograming. Knockdown of AID during nuclear reprograming by somatic cell fusion with ES cells leads to a failure to reactivate Oct4 and Nanog, possibly because of defective DNA demethylation at their promoters [50••]. Future work using RNAi screens will likely elucidate other molecular complexes involved in reprograming toward pluripotency *in vitro*.

While studies of the epigenetic regulation of iPS cell generation are expected to have broad application in regenerative medicine, they may in addition reveal potential molecular parallels with physiological reprograming *in vivo* [51]. In the next sections we describe some of these potential parallels, focusing on recent insights into epigenetic reprograming in the zygote and mid-gestation germ cells (Figure 1).

Epigenetic reprograming toward totipotency in the zygote

The zygote marks the starting point of development, and represents the reacquisition of totipotency from fusion of two highly differentiated gametes. The two parental genomes in the zygote have highly asymmetric chromatin organization [52,53]. Most histones are stripped from the paternal genome during spermatogenesis and replaced with highly basic protamines that allow for a very tight compaction of DNA [54–56]. A restricted set of nucleosomes is retained in about 4% of the genome, preferentially at developmental genes [57•]. These histones appear to be enriched in modifications like H3K27me3 and H3K4me3 in patterns that overlap significantly with those in ES cells. It thus appears that sperm DNA, while densely packed with protamines, may also transmit epigenetic marks important for early development [57•].

Shortly after fertilization, H3.3 is preferentially incorporated into the male pronucleus [53,58], presumably by maternal stores of chromatin factors that complement the transcriptionally silent zygote [59–61]. Although these factors have not been characterized in the mammalian zygote, maternal Chd1 has been shown to be required for incorporation of H3.3 into the male pronucleus in the *Drosophila* embryo [24]. *Drosophila* embryos derived from Chd1-null mothers exhibit a loss of the paternal genome and the resulting haploid embryos arrest before hatching. Functional studies of Chd1 in mouse should reveal whether its role in H3.3 incorporation in the male pronucleus is conserved, and if so, what the significance of this process is for mammalian development (Figure 1).

Another important epigenetic asymmetry between the parental genomes is that paternal (but not maternal) DNA is actively demethylated immediately following fertilization [62,63]. No DNA demethylase has been described despite extensive efforts [64]. With the aid of a live cell imaging reporter system, a recent RNAi screen implicated the transcription elongator complex in paternal DNA demethylation [65••]. The molecular mechanism behind elongator-mediated DNA demethylation needs to be further explored. In addition, it will be important to determine whether this represents a unique DNA

demethylation mechanism, or whether AID is involved. Finally, the relationship between H3.3 incorporation and DNA demethylation, both of which occur before the first cell division, remains to be explored.

Epigenetic reprograming in primordial germ cells

Another context where extensive chromatin remodeling occurs *in vivo* that may have parallels with reprograming *in vitro* is in primordial germ cells (PGCs). PGCs are specified by inductive signals around the time of gastrulation and represent the only lineage from the epiblast that actively represses the somatic cell fate in order to form oocytes and sperm later in development (reviewed in [66]). PGCs are also the only embryonic cells post-gastrulation that can still give rise to pluripotent stem cells when cultured *in vitro* [67]. These observations, coupled with the large number of regulators of ES cell pluripotency that are expressed in PGCs [68], suggest that some of same molecular mechanisms that maintain pluripotency *in vitro* may operate in PGCs.

Several recent studies [69••,70,71] paint an intricate picture of the dynamic epigenetic reprograming that takes place *in vivo* during PGC maturation. PGCs experience a large-scale loss of DNA methylation [72,73,74••] and many histone marks, including H3K9Ac, H3K9me3 and H3K27me3, around E11.5. Concurrently, linker histone H1 staining is lost and DAPI-stained chromatin becomes noticeably 'loosened'. Subsequently by E12.5, the transient loosening of the chromatin is reversed with the return of brightly stained DAPI foci, histone H1, H3K9me3, H3K27me3, and pericentromeric heterochromatin marks [69••].

It is possible that the rapid chromatin opening in E11.5 PGCs results from a large-scale incorporation of H3.3. In support of this hypothesis, the histone chaperone HIRA, which is essential for delivering H3.3 to active and repressed genes [18,75], is enriched in the nucleus of E11.5 PGCs [18,69••]. Chd1, which directly facilitates H3.3 deposition [24], is highly expressed in E11.5 PGCs [68]. Finally, H3.3 is essential for chromatin remodeling in the *Drosophila* germline, with both male and female flies mutant for H3.3 being sterile [76,77]. Altogether, it is tempting to speculate that maintenance of germline pluripotency, from flies to mice, requires the deposition of H3.3 by Chd1. It will therefore be of great interest to determine the function of Chd1, HIRA and H3.3 during mammalian PGC development (Figure 1).

The functional significance of this large-scale chromatin remodeling in PGCs remains unclear, but it may limit the transmission of epigenetic information across generations. It may not be a coincidence that, as in the case of the zygote, extensive chromatin remodeling in PGCs coincides with large-scale DNA demethylation [73]. Careful observations suggest that DNA demethylation may precede chromatin remodeling in PGCs [69••], but this remains to be demonstrated functionally. Intriguingly, AID was recently shown to be essential for efficient DNA demethylation in PGCs [74••], highlighting another potential parallel between reprograming in PGCs and *in vitro*.

Perspectives and future directions

Increasing evidence supports the notion that an open, decondensed chromatin state plays a vital role in the regulation of pluripotency in stem cells *in vitro* and during critical events of mammalian embryogenesis, including zygote and PGC development. The ease with which ES and iPS cells can be obtained in large numbers allows the application of unbiased genome-wide approaches such as ChIP-seq and RNAi screens. The integration of data from these approaches should shed light on the epigenetic architecture of the pluripotent stem cell state, and how it is reconfigured during differentiation. The process of reprograming to iPS cells remains largely a black box, and future studies are likely to reveal the epigenetic steps undertaken by somatic cells on the way to the open pluripotent chromatin state. The limited numbers of zygotes and PGCs and their rapidly changing transcriptional and epigenetic states during embryonic development pose significant technical hurdles to genome-wide analyses. Nevertheless, recent advancements have made genome-wide studies of chromatin states feasible for limited numbers of primary cells, and we expect that novel insights will be gained from applying these methods to pluripotent cells *in vivo*. In addition, candidate gene approaches that determine the *in vivo* roles of regulators of the open chromatin state of pluripotent stem cells, such as Chd1, may reveal common themes in the regulation of open chromatin. Research in the years ahead will likely reveal fascinating insights into the epigenetic regulation of pluripotency *in vitro* and its significance *in vivo*.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Chambers I, Silva J, Colby D, Nichols J, Nijmeijer B, Robertson M, Vrana J, Jones K, Grotewold L, Smith A: **Nanog safeguards pluripotency and mediates germline development**. *Nature* 2007, **450**:1230-1234.

2. Toyooka Y, Shimosato D, Murakami K, Takahashi K, Niwa H: **Identification and characterization of subpopulations in undifferentiated ES cell culture**. *Development* 2008, **135**:909-918.

3. Cui L, Johkura K, Yue F, Ogiwara N, Okouchi Y, Asanuma K, Sasaki K: **Spatial distribution and initial changes of SSEA-1 and other cell adhesion-related molecules on mouse embryonic stem cells before and during differentiation**. *J Histochem Cytochem* 2004, **52**:1447-1457.

4. Furusawa T, Ohkoshi K, Honda C, Takahashi S, Tokunaga T: **Embryonic stem cells expressing both platelet endothelial cell adhesion molecule-1 and stage-specific embryonic antigen-1 differentiate predominantly into epiblast cells in a chimeric embryo**. *Biol Reprod* 2004, **70**:1452-1457.

5. •• Hayashi K, Lopes SM, Tang F, Surani MA: **Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states**. *Cell Stem Cell* 2008, **3**:391-401.
The authors describe a subpopulation of cells within ES cell cultures that more closely resembles epiblast cells rather than ICM cells, suggesting heterogeneity within ES cells.

6. Bao S, Tang F, Li X, Hayashi K, Gillich A, Lao K, Surani MA: **Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells**. *Nature* 2009, **461**:1292-1295.

7. Cai S, Lee CC, Kohwi-Shigematsu T: **SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes**. *Nat Genet* 2006, **38**:1278-1288.

8. Dobreva G, Chahrour M, Dautzenberg M, Chirivella L, Kanzler B, Fariñas I, Karsenty G, Grosschedl R: **SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation**. *Cell* 2006, **125**:971-986.

9. Savarese F, Davila A, Nechanitzky R, De La Rosa-Velazquez I, Pereira CF, Engelke R, Takahashi K, Jenuwein T, Kohwi-Shigematsu T, Fisher AG *et al.*: **Satb1 and Satb2 regulate embryonic stem cell differentiation and Nanog expression**. *Genes Dev* 2009, **23**:2625-2638.

10. Efroni S, Duttagupta R, Cheng J, Dehghani H, Hoeppner DJ, Dash C, Bazett-Jones DP, Le Grice S, McKay RD, Buetow KH *et al.*: **Global transcription in pluripotent embryonic stem cells**. *Cell Stem Cell* 2008, **2**:437-447.

11. Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T: **Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells**. *Dev Cell* 2006, **10**:105-116.

12. Carter MG, Sharov AA, VanBuren V, Dudekula DB, Carmack CE, Nelson C, Ko MS: **Transcript copy number estimation using a mouse whole-genome oligonucleotide microarray**. *Genome Biol* 2005, **6**:R61.

13. • Hawkins RD, Hon GC, Lee LK, Ngo Q, Lister R, Pelizzola M, Edsall LE, Kuan S, Luu Y, Klugman S *et al.*: **Distinct epigenomic landscapes of pluripotent and lineage-committed human cells**. *Cell Stem Cell* 2010, **6**:479-491.
ChIP-seq for 11 chromatin marks shows that most changes in chromatin state between human ES cells and differentiated cells arise from the expansion of repressive H3K9me3 and H3K27me3 marks upon differentiation.

14. •• Gaspar-Maia A, Alajem A, Polesso F, Sridharan R, Mason MJ, Heidersbach A, Ramalho-Santos J, McManus MT, Plath K, Meshorer E *et al.*: **Chd1 regulates open chromatin and pluripotency of embryonic stem cells**. *Nature* 2009, **460**:863-868.
This paper shows that the chromatin remodeler Chd1 is required for open chromatin and pluripotency of mouse ES cells, and for somatic cell reprograming to the iPS cell state.

15. Sims RJ, Chen C-F, Santos-Rosa H, Kouzarides T, Patel SS, Reinberg D: **Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains**. *J Biol Chem* 2005, **280**:41789-41792.

16. Stokes DG, Tartof KD, Perry RP: **CHD1 is concentrated in interbands and puffed regions of Drosophila polytene chromosomes**. *Proc Natl Acad Sci U S A* 1996, **93**:7137-7142.

17. Persson J, Ekwall K: **Chd1 remodelers maintain open chromatin and regulate the epigenetics of differentiation**. *Exp Cell Res* 2010, **316**:1316-1323.

18. Goldberg AD, Banaszynski LA, Noh K-M, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, Guo X, Li X *et al.*: **Distinct factors control histone variant H3.3 localization at specific genomic regions**. *Cell* 2010, **140**:678-691.

19. Chow CM, Georgiou A, Szutorisz H, Maia e Silva A, Pombo A, Barahona I, Dargelos E, Canzonetta C, Dillon N: **Variant histone H3.3 marks promoters of transcriptionally active genes during mammalian cell division**. *EMBO Rep* 2005, **6**:354-360.

20. Mito Y, Henikoff JG, Henikoff S: **Genome-scale profiling of histone H3.3 replacement patterns**. *Nat Genet* 2005, **37**:1090-1097.

21. Schwartz BE, Ahmad K: **Transcriptional activation triggers deposition and removal of the histone variant H3.3**. *Genes Dev* 2005, **19**:804-814.

22. Braunschweig U, Hogan GJ, Pagie L, van Steensel B: **Histone H1 binding is inhibited by histone variant H3.3**. *EMBO J* 2009, **28**:3635-3645.

23. Elsaesser SJ, Goldberg AD, Allis CD: **New functions for an old variant: no substitute for histone H3.3**. *Curr Opin Genet Dev* 2010, **20**:110-117.

24. Konev AY, Tribus M, Park SY, Podhraski V, Lim CY, Emelyanov AV, Vershilova E, Pirrotta V, Kadonaga JT, Lusser A *et al.*: **CHD1 motor protein is required for deposition of histone variant H3.3 into chromatin in vivo**. *Science* 2007, **317**:1087-1090.

25. •• Ho L, Ronan JL, Wu J, Staahl BT, Chen L, Kuo A, Lessard J, Nesvizhskii AI, Ranish J, Crabtree GR: **An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency**. *Proc Natl Acad Sci U S A* 2009, **106**:5181-5186.
See annotation in Ref. [26••].

26. •• Ho L, Jothi R, Ronan JL, Cui K, Zhao K, Crabtree GR: **An embryonic stem cell chromatin remodeling complex, esBAF, is an essential component of the core pluripotency transcriptional network**. *Proc Natl Acad Sci U S A* 2009, **106**:5187-5191.
Along with Ref. [25••] this study demonstrates esBAF as an ES cell-specific chromatin remodeling complex that interacts with pluripotency-associated transcription factors and is required for maintenance of pluripotency.

27. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK *et al.*: **Polycomb complexes repress developmental regulators in murine embryonic stem cells**. *Nature* 2006, **441**:349-353.

28. Ku M, Koche RP, Rheinbay E, Mendenhall EM, Endoh M, Mikkelsen TS, Presser A, Nusbaum C, Xie X, Chi AS *et al.*: **Genomewide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains**. *PLoS Genet* 2008, **4**:e1000242.

29. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP *et al.*: **Genome-wide maps of chromatin state in pluripotent and lineage-committed cells**. *Nature* 2007, **448**:553-560.

30. O'Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, Jenuwein T: **The polycomb-group gene Ezh2 is required for early mouse development**. *Mol Cell Biol* 2001, **21**:4330-4336.

31. Wang J, Mager J, Schnedier E, Magnuson T: **The mouse PcG gene eed is required for Hox gene repression and extraembryonic development**. *Mamm Genome* 2002, **13**:493-503.

32. Voncken JW, Roelen BA, Roefs M, de Vries S, Verhoeven E, Marino S, Deschamps J, van Lohuizen M: **Rnf2 (Ring1b) deficiency causes gastrulation arrest and cell cycle inhibition**. *Proc Natl Acad Sci U S A* 2003, **100**:2468-2473.

33. Leeb M, Pasini D, Novatchkova M, Jaritz M, Helin K, Wutz A: **Polycomb complexes act redundantly to repress genomic repeats and genes**. *Genes Dev* 2010, **24**:265-276.

34. •• Peng JC, Valouev A, Swigut T, Zhang J, Zhao Y, Sidow A, Wysocka J: **Jarid2/Jumonji coordinates control of PRC2 enzymatic activity and target gene occupancy in pluripotent cells**. *Cell* 2009, **139**:1290-1302.
See annotation in Ref. [35••].

35. •• Shen X, Kim W, Fujiwara Y, Simon MD, Liu Y, Mysliwiec MR, Yuan GC, Lee Y, Orkin SH: **Jumonji modulates polycomb activity and self-renewal versus differentiation of stem cells**. *Cell* 2009, **139**:1303-1314.
Along with Ref. [34••] this study identifies Jarid2 as a core component of the PRC2 complex in ES cells that regulates targeting and activity of the complex.

36. Walker E, Chang WY, Hunkapiller J, Cagney G, Garcha K, Torchia J, Krogan NJ, Reiter JF, Stanford WL: **Polycomb-like 2 associates with PRC2 and regulates transcriptional networks during mouse embryonic stem cell self-renewal and differentiation**. *Cell Stem Cell* 2010, **6**:153-166.

37. Laurent L, Wong E, Li G, Huynh T, Tsirigos A, Ong CT, Low HM, Kin Sung KW, Rigoutsos I, Loring J *et al.*: **Dynamic changes in the human methylome during differentiation**. *Genome Res* 2010, **20**:320-331.

38. • Lister R, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo Q-M *et al.*: **Human DNA methylomes at base resolution show widespread epigenomic differences**. *Nature* 2009, **462**:315-322.
Genome wide DNA methylation map reveals an enrichment for non-CG methylation in human ES cells compared to a differentiated cell type.

39. Jones PA: **The DNA methylation paradox**. *Trends Genet* 1999, **15**:34-37.

40. Takahashi K, Yamanaka S: **Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors**. *Cell* 2006, **126**:663-676.

41. Chin MH, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, Ambartsumyan G, Aimiuwu O, Richter L, Zhang J *et al.*: **Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures**. *Cell Stem Cell* 2009, **5**:111-123.

42. • Mikkelsen TS, Hanna J, Zhang X, Ku M, Wernig M, Schorderet P, Bernstein BE, Jaenisch R, Lander ES, Meissner A: **Dissecting direct reprogramming through integrative genomic analysis**. *Nature* 2008, **454**:49-55.
One of the initial studies demonstrating the epigenetic similarities between ES and iPS cells. Partially reprogramed cells show incomplete DNA demethylation and ectopic expression of lineage-specific genes, which can be overcome with chemical inhibitors or RNAi.

43. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R *et al.*: **Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution**. *Cell Stem Cell* 2007, **1**:55-70.

44. Sridharan R, Tchieu J, Mason MJ, Yachechko R, Kuoy E, Horvath S, Zhou Q, Plath K: **Role of the murine reprogramming factors in the induction of pluripotency**. *Cell* 2009, **136**:364-377.

45. Meissner A, Wernig M, Jaenisch R: **Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells**. *Nat Biotechnol* 2007, **25**:1177-1181.

46. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA: **Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds**. *Nat Biotechnol* 2008, **26**:795-797.

47. Blelloch R, Wang Z, Meissner A, Pollard S, Smith A, Jaenisch R: **Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the donor nucleus**. *Stem Cells* 2006, **24**:2007-2013.

48. Simonsson S, Gurdon J: **DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei**. *Nat Cell Biol* 2004, **6**:984-990.

49. Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, Cairns BR: **DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45**. *Cell* 2008, **135**:1201-1212.

50. •• Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM: **Reprogramming towards pluripotency requires AID-dependent DNA demethylation**. *Nature* 2010, **463**:1042-1047.
AID is shown to be essential for active DNA demethylation during reprograming by somatic cell fusion of mouse ES cells and human fibroblasts.

51. Ramalho-Santos M: **iPS cells: insights into basic biology**. *Cell* 2009, **138**:616-618.

52. Puschendorf M, Terranova R, Boutsma E, Mao X, Isono K-i, Brykczynska U, Kolb C, Otte AP, Koseki H, Orkin SH *et al.*: **PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos**. *Nat Genet* 2008, **40**:411-420.

53. van der Heijden GW, Dieker JW, Derijck AA, Muller S, Berden JH, Braat DD, van der Vlag J, de Boer P: **Asymmetry in histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote**. *Mech Dev* 2005, **122**:1008-1022.

54. Balhorn R: **The protamine family of sperm nuclear proteins**. *Genome Biol* 2007, **8**:227.

55. Ward WS, Coffey DS: **DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells**. *Biol Reprod* 1991, **44**:569-574.

56. Wykes SM, Krawetz SA: **The structural organization of sperm chromatin**. *J Biol Chem* 2003, **278**:29471-29477.

57. • Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR: **Distinctive chromatin in human sperm packages genes for embryo development**. *Nature* 2009, **460**:473-478.
High-resolution genomic approaches show that some nucleosomes are retained at developmental genes in mature sperm and may contain epigenetic information important for early development.

58. Torres-Padilla ME, Bannister AJ, Hurd PJ, Kouzarides T, Zernicka-Goetz M: **Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos**. *Int J Dev Biol* 2006, **50**:455-461.

59. Latham KE, Solter D, Schultz RM: **Acquisition of a transcriptionally permissive state during the 1-cell stage of mouse embryogenesis**. *Dev Biol* 1992, **149**:457-462.

60. Levey IL, Stull GB, Brinster RL: **Poly(A) and synthesis of polyadenylated RNA in the preimplantation mouse embryo**. *Dev Biol* 1978, **64**:140-148.

61. Schultz RM: **The molecular foundations of the maternal to zygotic transition in the preimplantation embryo**. *Hum Reprod Update* 2002, **8**:323-331.

62. Barton SC, Arney KL, Shi W, Niveleau A, Fundele R, Surani MA, Haaf T: **Genome-wide methylation patterns in normal and uniparental early mouse embryos**. *Hum Mol Genet* 2001, **10**:2983-2987.

63. Oswald J, Engemann S, Lane N, Mayer W, Olek A, Fundele R, Dean W, Reik W, Walter J: **Active demethylation of the paternal genome in the mouse zygote**. *Curr Biol* 2000, **10**:475-478.

64. Ooi SKT, Bestor TH: **The colorful history of active DNA demethylation**. *Cell* 2008, **133**:1145-1148.

65. •• Okada Y, Yamagata K, Hong K, Wakayama T, Zhang Y: **A role for the elongator complex in zygotic paternal genome demethylation**. *Nature* 2010, **463**:554-558.
Using an siRNA screen and live zygote imaging, the authors identify the elongator complex as the novel player essential for paternal genome demethylation.

66. Sasaki H, Matsui Y: **Epigenetic events in mammalian germ-cell development: reprogramming and beyond**. *Nat Rev Genet* 2008, **9**:129-140.

67. Resnick JL, Bixler LS, Cheng L, Donovan PJ: **Long-term proliferation of mouse primordial germ cells in culture**. *Nature* 1992, **359**:550-551.

68. Grskovic M, Chaivorapol C, Gaspar-Maia A, Li H, Ramalho-Santos M: **Systematic identification of *cis*-regulatory sequences active in mouse and human embryonic stem cells**. *PLoS Genet* 2007, **3**:e145.

69. •• Hajkova P, Ancelin K, Waldmann T, Lacoste N, Lange UC, Cesari F, Lee C, Almouzni G, Schneider R, Surani MA: **Chromatin dynamics during epigenetic reprogramming in the mouse germ line**. *Nature* 2008, **452**:877-881.
This study describes the distinctive chromatin modifications that take place during PGC development and suggests that large-scale histone

replacement involving H3.3 and H2A.Z may be critical for the rapid changes observed.

70. Seki Y, Yamaji M, Yabuta Y, Sano M, Shigeta M, Matsui Y, Saga Y, Tachibana M, Shinkai Y, Saitou M: **Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice**. *Development* 2007, **134**:2627-2638.

71. Yabuta Y, Kurimoto K, Ohinata Y, Seki Y, Saitou M: **Gene expression dynamics during germline specification in mice identified by quantitative single-cell gene expression profiling**. *Biol Reprod* 2006, **75**:705-716.

72. Lee J, Inoue K, Ono R, Ogonuki N, Kohda T, Kaneko-Ishino T, Ogura A, Ishino F: **Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells**. *Development* 2002, **129**:1807-1817.

73. Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA: **Epigenetic reprogramming in mouse primordial germ cells**. *Mech Dev* 2002, **117**:15-23.

74. •• Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M, Jacobsen SE, Reik W: **Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency**. *Nature* 2010, **463**:1101-1105.
This study reveals that AID deficiency causes an increase in genome-wide DNA methylation in E13.5 PGCs but not in nongermline tissues, implying a PGC-specific role for AID in DNA demethylation.

75. Ahmad K, Henikoff S: **The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly**. *Mol Cell* 2002, **9**:1191-1200.

76. Sakai A, Schwartz BE, Goldstein S, Ahmad K: **Transcriptional and developmental functions of the H3.3 histone variant in Drosophila**. *Curr Biol* 2009, **19**:1816-1820.

77. Hödl M, Basler K: **Transcription in the absence of histone H3.3**. *Curr Biol* 2009, **19**:1221-1226.