REVIEW

Epigenetics in embryonic stem cells: regulation of pluripotency and differentiation

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Abstract Embryonic stem cells (ESC) are pluripotent cells capable of multilineage differentiation into every somatic cell type and therefore must be able to initiate transcription profiles for each of these different cells while maintaining their pluripotent state. Numerous studies have attempted to uncover the basic biology of the ESC and delineate mechanisms of pluripotency, but with limited success. However, recent studies attempting to understand the mechanisms by which gene expression is regulated in ESC have uncovered potential mechanisms that may be vitally important to their pluripotent nature. Epigenetic regulatory mechanisms include covalent modification of histone modification and DNA methylation. Studies have now shown that epigenetic mechanisms are vitally important to the pluripotent nature of ESC and that these mechanisms regulate differentiation. The epigenetic nature of the ESC has been demonstrated to be unique and its characteristics have been strongly linked to the global permissivity of gene expression and pluripotency. Unique mechanisms have also been suggested for the dynamic regulation of differentiation-associated gene expression. Additionally, epigenetic mechanisms have been indicated to play a role in the formation of the pluripotent cells of the inner cell mass in the developing blastocyst.

Keywords Histone modifications · Chromatin · Pluripotency · Embryonic stem cells · Polycomb

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Introduction

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of day 5–8 blastocysts (Thomson et al. 1998; Stojkovic et al. 2004) or morula-stage embryos (Strelchenko et al. 2004). ESC are pluripotent, are capable of karyotypically stable prolonged self-renewal and are characterised by their potential to differentiate into cells of the three germ layers, both in vitro and in vivo (Evans and Kaufman 1981; Thomson et al. 1998; Stojkovic et al. 2004). In contrast to the specific gene expression programmes observed in differentiated cells, ESC are defined by their potential to activate all of the gene expression programmes that are found in embryonic and adult cell lineages (Chambers and Smith 2004). Various studies have endeavoured to understand the molecular mechanisms that give ESC their properties (Ivanova et al. 2002; Ramalho-Santos et al. 2002; Sperger et al. 2003; Margueron et al. 2005; Armstrong et al. 2006a, b) and these attempts to identify a molecular "signature" of ESC have borne some fruit, including the identification of the transcription factors OCT4 and NANOG as markers for ESC.

However, these experiments also show that there is little other overlap in terms of gene expression between different ESC lines and also within the same ESC lines studied in different laboratories. This lack of overlap questions whether a common molecular identity of ESC can be uncovered (Evsikov and Solter 2003; Fortunel et al. 2003; Vogel 2003). However, recent studies have indicated that epigenetic mechanisms, processes that can alter gene function without a change in the DNA sequence, may regulate self-renewal, pluripotency and lineage-specific differentiation, giving ESC their unique characteristics. Epigenetic mechanisms include modification of histones proteins, DNA methylation, ATP-dependent remodelling,

incorporation of variant histones, changes in local and higher order conformation of DNA, and RNA interference (RNAi). Through the combined efforts of these epigenetic mechanisms, gene expression patterns can be tightly and dynamically regulated.

Chromatin modification in ESC pluripotency and differentiation

Various observations have suggested that the genome undergoes important epigenetic alterations during mammalian development and ESC differentiation (Margueron et al. 2005; Morgan et al. 2005; Reik 2007), from an open environment rich in euchromatin in ESC, to a more compact heterochromatic structure upon differentiation (Francastel et al. 2000; Arney and Fisher 2004). Somatic cell nuclear transfer (SCNT) experiments have also demonstrated that reprogramming of the somatic nucleus to a pluripotent state requires large-scale epigenetic modifications (Armstrong et al. 2006a, b). Several groups have now begun to understand the role of epigenetics in the ESC and have suggested some interesting models.

The chromatin structure of mouse ESC (mESC) has now been demonstrated to be hyperdynamic, in that major architectural proteins, such as $H1^0$, H2B, H3 and HP1α, are loosely bound to chromatin with extremely short residency times (Meshorer et al. 2006). Differentiation leads to a decrease in the dynamic nature of these proteins, as demonstrated by increased residency time, suggesting that this dynamic nature of chromatin is specific to pluripotent cells and that differentiation leads to the restructuring of the genome. Additionally, heterochromatic markers have been demonstrated to change from a dispersed localisation in mESC, to more concentrated distinct foci in differentiated cells, with increased global levels of trimethylated lysine 9 H3 (TriMeK9 H3), a heterochromatic histone modification linked to gene repression, and decreased levels of acetylated histones H3 and H4 (AcH3 and AcH4), modifications linked to euchromatin and the permissivity of gene expression. Analyses of global histone modification patterns in ESC has previously suggested that the ESC genome is subject to generalised histone acetylation and lysine 4 H3 methylation (MeK4 H3; Kimura et al. 2004; Lee et al. 2004). Therefore, these changes in global genomic architecture and global histone modifications suggest that the chromatin environment in ESC is highly euchromatic and that the genome is therefore highly permissive for gene expression. This would allow for pluripotent nature of ESCs, with the genome becoming more structured, condensed and heterochromatic upon differentiation, leading to loss of pluripotency.

Another study on mESC has aimed at understanding regulatory mechanisms involved in development by studying highly conserved noncoding elements (HCNEs; Bernstein et al. 2006). These elements have been studied because of their presence in regions concentrated for genes encoding developmentally important transcription factors. Large-scale chromatin immunoprecipitation (ChIP) assays have found large areas of a repressive histone modification, methylation of Lysine 27 histone H3 (MeK27 H3; Cao and Zhang 2004), along-side smaller regions of a permissive modification, MeK4 H3 (Schubeler et al. 2004; Bernstein et al. 2005) at HCNEs. This is unusual insomuch that these modifications are generally mutually exclusive; hence, these regions have been termed "bivalent" domains. Interestingly, the bivalent domains coincide with differentiation-associated transcription factor genes expressed at extremely low levels in the ESC and they have therefore been proposed to act not only to silence such genes in order to maintain pluripotency, but also to allow them to remain poised for transcription, so they can be rapidly activated on differentiation. Upon differentiation, these bivalent domains "resolve", so that silent genes become concentrated for MeK27 H3 only and active genes become enriched for euchromatic modifications. The large regions that the bivalent domains cover may also provide a means of transmitting epigenetic modifications through cell division ("epigenetic memory"; Henikoff et al. 2004; van Steensel 2005) to maintain lineage-specific expression or repression of such critical genes. This would be important for the transmission of such regulatory mechanisms through differentiation and replication towards somatic cells. Approximately half of the identified bivalent domains have been found to have binding sites for at least one of the three pluripotency-associated transcription factors, viz. OCT4, NANOG and SOX2 (Boyer et al. 2005), meaning that these silenced genes are associated with pluripotent transcription factors normally involved with gene activation (Boiani and Scholer 2005; Stewart et al. 2006). NANOG, SOX2 and OCT4 are known to be able to interact with epigenetic modifiers, including Polycomb Group (PcG) proteins, and therefore may have a role to play in modifying the epigenetic environment of gene promoters (Wang et al. 2006).

Perhaps most interestingly, the study (Bernstein et al. 2006) has also found a correlation between the chromatin environment and the underlying DNA sequence, suggesting a mechanism by which the DNA sequence can dictate histone modification patterns. A striking correlation exists between the presence of MeK4 H3 and CpG islands and the transcription start sites in ESCs. MeK27 H3 is correlated with the lack of transposon-derived sequence and such regions have been further shown to be enriched at genes encoding developmental and tissue-specific transcription factors. This mechanism would provide a means for the early chromatin environment to be organised efficiently and suitably for the purpose of pluripotency in the ESC.

Similar studies into the epigenetic basis of ESC pluripotency and differentiation have uncovered a role for PcG proteins. Genetic experiments in *Drosophila* have demonstrated that PcG members are required for maintaining the inactive state of homeotic and other important regulators during development (Ringrose and Paro 2004). They have also been shown to be required for early embryo development (Voncken et al. 2003; Pasini et al. 2004; Isono et al. 2005) and have been previously implicated in ESC pluripotency (O'Carroll et al. 2001). The first study utilised replication time at S-phase as an indicator of the chromatic state of the genome (euchromatic accessible genes replicate earlier, heterochromatic condensed genes later; Schubeler et al. 2002; Azuara et al. 2003) and compared ESC and differentiated cells (Azuara et al. 2006). Surprisingly, silent-lineage-associated genes were transcribed at the same time as pluripotency-associated genes in ESC, indicating similarities in their chromatin environment. However, upon differentiation, pluripotency-associated genes changed their replication timing to later, whereas lineage-associated genes replicated earlier. The finding that lineage-associated genes replicate early in ESC alongside pluripotency-associated genes suggests that they have a permissive chromatin structure while remaining silent. Further studies have demonstrated that the silent-lineage-associated genes also have the "bivalent" chromatin structure observed previously. The functionality of the MeK27 H3 modification has been demonstrated in ESC lacking *EED*, a member of the PRC2 PcG complex, which is required for EZH2-mediated K27 H3 methylation (Kirmizis et al. 2004); these cells show premature expression of previously silenced genes and loss of pluripotency.

The role of MeK27 H3 and its link to pluripotency in ESC has been further analysed in another study of mESC. Similar to the previous studies, TriMeK27 H3 has been found at the promoters of silent-lineage-associated genes in mESC; this is associated with the binding of components of PRC1 and PRC2 PcG complexes (Boyer et al. 2006). Another study has also revealed that the binding sites for the PcG group protein SUZ12 (Lee et al. 2006) are associated with developmentally important transcription factor genes, HCNEs, TriMeK27 H3 and gene repression. Again, OCT4, NANOG and SOX2 also bind a high percentage of these genes. Upon differentiation, such genes are specifically upregulated, with the loss of TriMeK27 H3, suggesting that PcG proteins have specialised roles in silencing genes in ESC. Again, the functionality of MeK27 H3 has been shown by the lack of repression of lineage-associated gene expression in *EED*-deficient cells (Boyer et al. 2006). Additionally, SUZ12 binding has been assayed in differentiated somatic cells (Lee et al. 2006). SUZ12 is depleted at transcriptional regulators required for the said somatic cell type, whereas SUZ12 is still found at promoters of regulators not required in the said cell type, supporting a model in which PRC2 binding in ESC represses key developmental regulators that are later expressed during differentiation.

PcG proteins have also been linked to DNA methylation (Vire et al. 2006), which is in turn linked to gene silencing and alterations of chromatin structure (Lande-Diner et al. 2007). DNA methylation patterns change during differentiation, with lower DNA methylation, and therefore a more euchromatic and open chromatin environment, as is apparent in ESC (Shiota et al. 2002). Additionally, DNA methylation profiling has shown that ESC have a unique DNA methylation pattern that may contribute to pluripotency and development when compared with many other cell types (Bibikova et al. 2006). A model whereby the long term silencing of lineage-irrelevant genes upon differentiation could be directed by PcG proteins and given stability by DNA methylation is appealing (Shiota et al. 2002).

Chromatin bivalency provides an interesting mechanism to maintain pluripotency and regulate differentiation. However, some loci show no evidence for chromatin bivalency at the ESC stage (Anguita et al. 2004; Szutorisz et al. 2005). Szutorisz et al. (2005) have suggested another epigenetic model by which ESC can regulate pluripotency and differentiation; they have demonstrated that intergenic regions are marked by histone modifications in the ESC and aid in the temporal regulation of gene transcription (Szutorisz et al. 2005). Their study has concentrated on the λ5-VpreB1 locus in mESC and they have found that the intergenic region contains a discrete region of AcH3 and MeK4 H3 in the ESC. Further analysis has shown that, during B-cell development, the modifications spread from this discrete region, leading to the formation of a large active chromatin domain in pre-B cells (correlated to gene expression), whereas in non-haematopoietic cells, the modifications become erased (correlated to gene repression). These modifications also act as a nucleation point for general transcription factors and RNA polymerase II. Evidence for this regulatory mechanism has also been extended to some other genes (Chakrabarti et al. 2003; Chambeyron and Bickmore 2004; Vieira et al. 2004). This model suggests that the pluripotent state, viz. a state with large differentiation potential, would have more of these discrete regions of histone modifications (termed early transcription competence marks; ETCMs) than, for example, a hepatic stem cell, which is more restricted in terms of differentiation potential and which therefore would have fewer ETCMs.

Another suggested model for epigenetic regulation of the ESC is the histone "pulsing" model (Gan et al. 2007) in which each individual ESC exhibits variable histone modification patterns at individual genes over time. The model suggests that silent-lineage-associated genes, whose chromatin environment has been previously described as

bivalent, have a modification patterning that changes at a higher frequency and/or a longer duration, whereas those not showing a bivalent chromatin structure have a lower frequency and/or a shorter duration of change. This leads to the supposition that two mutually exclusive modifications exist at the same gene (as for bivalency) but that, really, the ChIP assay is giving a representation of two (or more) different chromatin states because of the use of a heterogeneous ESC population for the studies. Therefore, gene expression would only occur in a cell that had a permissive chromatin environment at the gene at the time of stimulation. This suggests that ESCs are a highly heterogeneous population, with regards to their chromatic environment, correlating with previous data demonstrating elevated dynamics of histone residence in ESC (Meshorer et al. 2006). It also correlates with data demonstrating that the pluripotent cells of the ICM are heterogeneous (Chazaud et al. 2006); this may be attributable to the different reactions of the pluripotent cells of the ICM to a stimulatory signal, caused by a heterogeneous chromatin environment and gene expression potential. However, Re-ChIP analysis has shown that MeK4 H3 and MeK27 H3 do occur at the same time on the same gene suggesting that bivalency is a *bona fide* regulatory mechanism (Bernstein et al. 2006). This does not however rule out this epigenetic model in ESC.

Chromatin remodelling and differentiation

Consistent with the notion of genomic re-organisation upon differentiation, numerous ATP-dependent chromatin remodellers are elevated in ESC (Kurisaki et al. 2005) and are required for differentiation (Kaji et al. 2007). The requirement for these proteins correlates with previous studies of the hyperdynamism of structural proteins (Meshorer et al. 2006). Loss of chromatin remodelling complex proteins has also been shown to lead to lethality at the blastocyst stage, when the ICM is being formed (Klochendler-Yeivin et al. 2000; Cao et al. 2003; Houlard et al. 2006), suggesting that some rearrangement must occur before the formation of the ICM and that epigenetics plays a role in the development of the pluripotent state.

Histone modifications influence formation of pluripotent ESC

Modifications to histones may also play a role in the initial formation of the pluripotent cell within the ICM of the mammalian embryo. Differing developmental fate and potency in the mouse embryo may be established as early as the four-cell stage (Fujimori et al. 2003; Piotrowska-Nitsche et al. 2005) and now Torres-Padilla et al. (2007)

have suggested that the regulation of histone modifications might play a key role in this mechanism. Arginine methylation of histone proteins has been linked to gene activation (Chen et al. 1999; Ma et al. 2001) and this study (Torres-Padilla et al. 2007) has found that levels of methylation of specific arginines are correlated with cell fate and potency. High methylation at the four-cell stage is correlated with a contribution to the ICM and polar trophectoderm with elevated levels of global transcription, whereas low levels of methylation are correlated with a contribution to the mural trophectoderm. Overexpression of CARM1, an arginine methyltransferase, in individual blastomeres directs these cells progeny to become part of the ICM and also results in a dramatic upregulation of NANOG and SOX2. Again, this study links an open chromatin environment, leading to permissivity of gene expression, with pluripotent potential and identifies specific histone modifications as the earliest known epigenetic markers contributing towards the development of pluripotent cells. It also indicates that the manipulation of epigenetic information can influence cell fate determination.

The ability to manipulate the "epigenotype" of cells may have far-reaching consequences, for if we can alter histone modifications at will, we may be able to reprogram somatic genomes to a more pluripotent state. Epigenetic reprogramming is of course the basis of SCNT, which is capable of repressing the unique repertoire of somatic cell gene products (Armstrong et al. 2006a, b); however, as a means of producing patient-specific pluripotent cell lines, SCNT has many drawbacks. The value of the technique lies in the investigation of the mechanisms by which epigenetic reprogramming occurs; our increasing knowledge of the impact of epigenetics on pluripotency and the newer technologies for examining epigenetic changes by using small cell numbers are laying the groundwork for such an investigation.

Exciting new research has perhaps provided a means of the generation of relatively large numbers of patient-specific pluripotent cells, by the transfection of just four defined factors into somatic cells (Takahashi and Yamanaka 2006). Although the exact details of just how these factors (Oct3/4, Sox2, Klf4 and Myc) reprogram normal cells into pluripotent cells are not fully understood, Myc is known to be able to induce widespread changes in the chromatin environment and to mediate immortalisation and has been suggested to be required for the other factors further to mediate the reprogramming process (Yamanaka 2007).

Testing ESC epigenetics in vivo: a problem solved?

Large-scale ChIP analyses have proved to be a vital tool in understanding the epigenetic mechanisms in cultured ESC cells. However, a conventional ChIP assay tends to use

cells in their millions for analysis, preventing its use for cells of the ICM, which can be as low as 20 cells. The employment of a modified ChIP assay with *Drosophila* SL2 cells as a "carrier" for ESC (carrier ChIP) has lowered the level of ESCs needed from millions to as low as 100 cells (O'Neill et al. 2006). This has allowed the study of histone modifications at key regulator genes in small amounts of ESCs and cells of the ICM and trophectoderm of cultured blastocysts. O'Neill et al. (2006) have used this assay and found that the promoter regions of *NANOG* and *POU5F1* (OCT3/4) are enriched for AcH4 and TriMeK4 H3 in the ICM, where they are active, but depleted in AcH4 and TriMeK4 H3 and enriched for MeK9 H3 in the trophectoderm, where they are silent, with the converse being found for *CDX2*, which is expressed in the trophectoderm. Carrier ChIP analyses of ICM cells and ESC correlate well, although the high levels of MeK9 H3 and low levels of AcH4 found in the ICM cells for silent genes are lower in ESC; carrier ChIP also correlates with typical ChIP analysis using ESC.

Nuclear architecture and pluripotency

Genomes are known to have a distinct structure within the cell nucleus (Parada and Misteli 2002) and different nuclear positioning can correlate with their functional output (Spector 2003). Generally, gene-rich chromosomes are located in the centre of the nucleus, whereas more gene-poor chromosomes localise towards the nuclear periphery (Gilbert et al. 2005; Sproul et al. 2005). Studies of the nuclear organisation of human ESC (hESC) has shown that the organisation of chromosomes in hESC is similar to that observed in differentiated cells but that certain chromosome regions and gene loci with a role in pluripotency have a distinct localisation (Wiblin et al. 2005). Chromosome 12p, which contains clustered pluripotency genes (including *NANOG*), has a central nuclear localisation in ESC relative to differentiated cells, whereas chromosome 6p shows no change in nuclear chromosome position, but the *OCT4* locus is relocalised to a position outside its chromosome territory. This has also been observed for the *HOXB* locus in mESC upon retinoic-acid-mediated differentiation (Chambeyron et al. 2005). These genes have been shown to be associated with high levels of permissive chromatin modifications and this may therefore link to the chromosomal territory (O'Neill et al. 2006).

RNAi and pluripotency

A further suggested model of the regulation of pluripotency and differentiation in ESC involves RNAi, which has roles in heterochromatin formation (Wassenegger 2005). More genes are observed to be expressed in ESC relative to differentiated cells (Abeyta et al. 2004; Eckfeldt et al. 2005) correlating well with the globally permissive chromatin environment observed. Non-coding sequences are also active in ESC (Lehnertz et al. 2003; Martens et al. 2005); this may be vital for heterochromatin formation by RNAi upon differentiation (Meshorer et al. 2006). Indeed, perturbations to the RNAi machinery in ESC disrupts differentiation (Kanellopoulou et al. 2005). Interestingly, PcG proteins have been shown not only to colocalise with ARGONAUTE1, a core component of the RNAi machinery, at target promoters (Kim et al. 2006), but also to possess RNA-binding properties (Zhang et al. 2004), suggesting that RNAi and PcG may work together to mediate heterochromatin formation upon differentiation.

Concluding remarks

Many interesting models and mechanisms have been suggested for the way that epigenetics impact ESC biology, but which model is utilised by ESCs? All the mechanisms suggested probably have some role to play and further studies might uncover the nature of the dynamic interaction between transcription factors, epigenetic regulators and other important effectors in the regulation of ESC biology.

References

Abeyta MJ, Clark AT, Rodriguez RT, Bodnar MS, Pera RA, Firpo MT (2004) Unique gene expression signatures of independently-derived human embryonic stem cell lines. Hum Mol Genet 13:601–608

Anguita E, Hughes J, Heyworth C, Blobel GA, Wood WG, Higgs DR (2004) Globin gene activation during haemopoiesis is driven by protein complexes nucleated by GATA-1 and GATA-2. EMBO J 23:2841–2852

Armstrong L, Hughes O, Yung S, Hyslop L, Stewart R, Wappler I, Peters H, Walter T, Stojkovic P, Evans J, Stojkovic M, Lako M (2006a) The role of PI3K/AKT, MAPK/ERK and NFkappabeta signalling in the maintenance of human embryonic stem cell pluripotency and viability highlighted by transcriptional profiling and functional analysis. Hum Mol Genet 15:1894–1913

Armstrong L, Lako M, Dean W, Stojkovic M (2006b) Epigenetic modification is central to genome reprogramming in somatic cell nuclear transfer. Stem Cells 24:805–814

Arney KL, Fisher AG (2004) Epigenetic aspects of differentiation. J Cell Sci 117:4355–4363

Azura V, Brown KE, Williams RR, Webb N, Dillon N, Festenstein R, Buckle V, Merkenschlager M, Fisher AG (2003) Heritable gene silencing in lymphocytes delays chromatid resolution without affecting the timing of DNA replication. Nat Cell Biol 5:668–674

Azura V, Perry P, Sauer S, Spivakov M, Jorgensen HF, John RM, Gouti M, Casanova M, Warnes G, Merkenschlager M, Fisher AG (2006) Chromatin signatures of pluripotent cell lines. Nat Cell Biol 8:532–538

Bernstein BE, Kamal M, Lindblad-Toh K, Bekiranov S, Bailey DK, Huebert DJ, McMahon S, Karlsson EK, Kulbokas EJ 3rd, Gingeras TR, Schreiber SL, Lander ES (2005) Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 120:169–181

Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326

Bibikova M, Chudin E, Wu B, Zhou L, Garcia EW, Liu Y, Shin S, Plaia TW, Auerbach JM, Arking DE, Gonzalez R, Crook J, Davidson B, Schulz TC, Robins A, Khanna A, Sartipy P, Hyllner J, Vanguri P, Savant-Bhonsale S, Smith AK, Chakravarti A, Maitra A, Rao M, Barker DL, Loring JF, Fan JB (2006) Human embryonic stem cells have a unique epigenetic signature. Genome Res 16:1075–1083

Boiani M, Scholer HR (2005) Regulatory networks in embryo-derived pluripotent stem cells. Nat Rev Mol Cell Biol 6:872–884

Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122:947–956

Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 441:349–353

Cao R, Zhang Y (2004) The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr Opin Genet Dev 14:155–164

Cao S, Bendall H, Hicks GG, Nashabi A, Sakano H, Shinkai Y, Gariglio M, Oltz EM, Ruley HE (2003) The high-mobility-group box protein SSRP1/T160 is essential for cell viability in day 3.5 mouse embryos. Mol Cell Biol 23:5301–5307

Chakrabarti SK, Francis J, Ziesmann SM, Garmey JC, Mirmira RG (2003) Covalent histone modifications underlie the developmental regulation of insulin gene transcription in pancreatic beta cells. J Biol Chem 278:23617–23623

Chambers I, Smith A (2004) Self-renewal of teratocarcinoma and embryonic stem cells. Oncogene 23:7150–7160

Chambeyron S, Bickmore WA (2004) Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. Genes Dev 18:1119–1130

Chambeyron S, Da Silva NR, Lawson KA, Bickmore WA (2005) Nuclear re-organisation of the Hoxb complex during mouse embryonic development. Development 132:2215–2223

Chazaud C, Yamanaka Y, Pawson T, Rossant J (2006) Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. Dev Cell 10:615–624

Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR (1999) Regulation of transcription by a protein methyltransferase. Science 284:2174–2177

Eckfeldt CE, Mendenhall EM, Verfaillie CM (2005) The molecular repertoire of the "almighty" stem cell. Nat Rev Mol Cell Biol 6:726–737

Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154–156

Evsikov AV, Solter D (2003) Comment on "'Stemness': transcriptional profiling of embryonic and adult stem cells" and "A stem cell molecular signature". Science 302:393

Fortunel NO, Otu HH, Ng HH, Chen J, Mu X, Chevassut T, Li X, Joseph M, Bailey C, Hatzfeld JA, Hatzfeld A, Usta F, Vega VB, Long PM, Libermann TA, Lim B (2003) Comment on "'Stemness': transcriptional profiling of embryonic and adult stem cells" and "A stem cell molecular signature". Science 302:393

Francastel C, Schubeler D, Martin DI, Groudine M (2000) Nuclear compartmentalization and gene activity. Nat Rev Mol Cell Biol 1:137–143

Fujimori T, Kurotaki Y, Miyazaki J, Nabeshima Y (2003) Analysis of cell lineage in two- and four-cell mouse embryos. Development 130:5113–5122

Gan Q, Yoshida T, McDonald OG, Owens GK (2007) Concise review: epigenetic mechanisms contribute to pluripotency and cell lineage determination of embryonic stem cells. Stem Cells 25:2–9

Gilbert N, Gilchrist S, Bickmore WA (2005) Chromatin organization in the mammalian nucleus. Int Rev Cytol 242:283–336

Henikoff S, Furuyama T, Ahmad K (2004) Histone variants, nucleosome assembly and epigenetic inheritance. Trends Genet 20:320–326

Houlard M, Berlivet S, Probst AV, Quivy JP, Hery P, Almouzni G, Gerard M (2006) CAF-1 is essential for heterochromatin organization in pluripotent embryonic cells. PLoS Genet 2:e181

Isono K, Fujimura Y, Shinga J, Yamaki M, OW J, Takihara Y, Murahashi Y, Takada Y, Mizutani-Koseki Y, Koseki H (2005) Mammalian polyhomeotic homologues Phc2 and Phc1 act in synergy to mediate polycomb repression of Hox genes. Mol Cell Biol 25:6694–6706

Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR (2002) A stem cell molecular signature. Science 298:601–604

Kaji K, Nichols J, Hendrich B (2007) Mbd3, a component of the NuRD co-repressor complex, is required for development of pluripotent cells. Development 134:1123–1132

Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K (2005) Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev 19:489–501

Kim DH, Villeneuve LM, Morris KV, Rossi JJ (2006) Argonaute-1 directs siRNA-mediated transcriptional gene silencing in human cells. Nat Struct Mol Biol 13:793–797

Kimura H, Tada M, Nakatsuji N, Tada T (2004) Histone code modifications on pluripotential nuclei of reprogrammed somatic cells. Mol Cell Biol 24:5710–5720

Kirmizis A, Bartley SM, Kuzmichev A, Margueron R, Reinberg D, Green R, Farnham PJ (2004) Silencing of human polycomb target genes is associated with methylation of histone H3 Lys 27. Genes Dev 18:1592–1605

Klochendler-Yeivin A, Fiette L, Barra J, Muchardt C, Babinet C, Yaniv M (2000) The murine SNF5/INI1 chromatin remodeling factor is essential for embryonic development and tumor suppression. EMBO Rep 1:500–506

Kurisaki A, Hamazaki TS, Okabayashi K, Iida T, Nishine T, Chonan R, Kido H, Tsunasawa S, Nishimura O, Asashima M, Sugino H (2005) Chromatin-related proteins in pluripotent mouse embryonic stem cells are downregulated after removal of leukemia inhibitory factor. Biochem Biophys Res Commun 335:667–675

Lande-Diner L, Zhang J, Ben-Porath I, Amariglio N, Keshet I, Hecht M, Azuara V, Fisher AG, Rechavi G, Cedar H (2007) Role of DNA methylation in stable gene repression. J Biol Chem 282:12194–12200

Lee JH, Hart SR, Skalnik DG (2004) Histone deacetylase activity is required for embryonic stem cell differentiation. Genesis 38:32–38

Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA (2006) Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 125:301–313

Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, Chen T, Li E, Jenuwein T, Peters AH (2003) Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 13:1192–1200

Ma H, Baumann CT, Li H, Strahl BD, Rice R, Jelinek MA, Aswad DW, Allis CD, Hager GL, Stallcup MR (2001) Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. Curr Biol 11:1981–1985

Margueron R, Trojer P, Reinberg D (2005) The key to development: interpreting the histone code? Curr Opin Genet Dev 15:163–176

Martens JH, O'Sullivan RJ, Braunschweig U, Opravil S, Radolf M, Steinlein P, Jenuwein T (2005) The profile of repeat-associated histone lysine methylation states in the mouse epigenome. EMBO J 24:800–812

Meshorer E, Misteli T (2006) Chromatin in pluripotent embryonic stem cells and differentiation. Nat Rev Mol Cell Biol 7:540–546

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

Morgan HD, Santos F, Green K, Dean W, Reik W (2005) Epigenetic reprogramming in mammals. Hum Mol Genet 14 (Spec No 1): R47–R58

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

O'Neill LP, VerMilyea MD, Turner BM (2006) Epigenetic characterization of the early embryo with a chromatin immunoprecipitation protocol applicable to small cell populations. Nat Genet 38:835–841

Parada L, Misteli T (2002) Chromosome positioning in the interphase nucleus. Trends Cell Biol 12:425–432

Pasini D, Bracken AP, Jensen MR, Lazzerini Denchi E, Helin K (2004) Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. EMBO J 23:4061–4071

Piotrowska-Nitsche K, Perea-Gomez A, Haraguchi S, Zernicka-Goetz M (2005) Four-cell stage mouse blastomeres have different developmental properties. Development 132:479–490

Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA (2002) "Stemness": transcriptional profiling of embryonic and adult stem cells. Science 298:597–600

Reik W (2007) Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447:425–432

Ringrose L, Paro R (2004) Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu Rev Genet 38:413–443

Schubeler D, Scalzo D, Kooperberg C, Steensel B van, Delrow J, Groudine M (2002) Genome-wide DNA replication profile for Drosophila melanogaster: a link between transcription and replication timing. Nat Genet 32:438–442

Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F, Gottschling DE, O'Neill LP, Turner BM, Delrow J, Bell SP, Groudine M (2004) The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. Genes Dev 18: 1263–1271

Shiota K, Kogo Y, Ohgane J, Imamura T, Urano A, Nishino K, Tanaka S, Hattori N (2002) Epigenetic marks by DNA methylation specific to stem, germ and somatic cells in mice. Genes Cells 7:961–969

Spector DL (2003) The dynamics of chromosome organization and gene regulation. Annu Rev Biochem 72:573–608

Sperger JM, Chen X, Draper JS, Antosiewicz JE, Chon CH, Jones SB, Brooks JD, Andrews PW, Brown PO, Thomson JA (2003) Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. Proc Natl Acad Sci USA 100:13350–13355

Sproul D, Gilbert N, Bickmore WA (2005) The role of chromatin structure in regulating the expression of clustered genes. Nat Rev Genet 6:775–781

Steensel B van (2005) Mapping of genetic and epigenetic regulatory networks using microarrays. Nat Genet 37 Suppl:S18–S24

Stewart R, Stojkovic M, Lako M (2006) Mechanisms of self-renewal in human embryonic stem cells. Eur J Cancer 42:1257–1272

Stojkovic M, Lako M, Stojkovic P, Stewart R, Przyborski S, Armstrong L, Evans J, Herbert M, Hyslop L, Ahmad S, Murdoch A, Strachan T (2004) Derivation of human embryonic stem cells from day-8 blastocysts recovered after three-step in vitro culture. Stem Cells 22:790–797

Strelchenko N, Verlinsky O, Kukharenko V, Verlinsky Y (2004) Morula-derived human embryonic stem cells. Reprod Biomed Online 9:623–629

Szutorisz H, Canzonetta C, Georgiou A, Chow CM, Tora L, Dillon N (2005) Formation of an active tissue-specific chromatin domain initiated by epigenetic marking at the embryonic stem cell stage. Mol Cell Biol 25:1804–1820

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

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147

Torres-Padilla ME, Parfitt DE, Kouzarides T, Zernicka-Goetz M (2007) Histone arginine methylation regulates pluripotency in the early mouse embryo. Nature 445:214–218

Vieira KF, Levings PP, Hill MA, Crusselle VJ, Kang SH, Engel JD, Bungert J (2004) Recruitment of transcription complexes to the beta-globin gene locus in vivo and in vitro. J Biol Chem 279:50350–50357

Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM, Bollen M, Esteller M, Di Croce L, Launoit Y de, Fuks F (2006) The Polycomb group protein EZH2 directly controls DNA methylation. Nature 439: 871–874

Vogel G (2003) Stem cells. "Stemness" genes still elusive. Science 302:371

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

Wang J, Rao S, Chu J, Shen X, Levasseur DN, Theunissen TW, Orkin SH (2006) A protein interaction network for pluripotency of embryonic stem cells. Nature 444:364–368

Wassenegger M (2005) The role of the RNAi machinery in heterochromatin formation. Cell 122:13–16

Wiblin AE, Cui W, Clark AJ, Bickmore WA (2005) Distinctive nuclear organisation of centromeres and regions involved in pluripotency in human embryonic stem cells. J Cell Sci 118:3861–3868

Yamanaka S (2007) Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell 1:39–49

Zhang H, Christoforou A, Aravind L, Emmons SW, Heuvel S van den, Haber DA (2004) The C. elegans Polycomb gene SOP-2 encodes an RNA binding protein. Mol Cell 14:841–847