OPINION

Chromatin in pluripotent embryonic stem cells and differentiation

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Abstract | Embryonic stem (ES) cells are unique in that they are pluripotent and have the ability to self-renew. The molecular mechanisms that underlie these two fundamental properties are largely unknown. We discuss how unique properties of chromatin in ES cells contribute to the maintenance of pluripotency and the determination of differentiation properties.

Embryonic stem (ES) cells are pluripotent cells that are derived from the inner cell mass of the pre-implantation embryo at the blastocyst stage. They are characterized by their potential to self-renew indefinitely and to differentiate into any of the three germ layers — endoderm, mesoderm and ectoderm[1,2]. These key functional properties of ES cells place opposing constraints on their genome. Self-renewal requires that the ES-cell genome maintains a cellular memory that specifies its pluripotent capacity. On the other hand, the genome in pluripotent ES cells must be in a highly plastic state so as to have the capacity to enter any one distinct differentiation pathway. Once differentiation is initiated, lineage specification occurs by the implementation of genome-expression programmes that give each cell type a unique transcriptional profile. The molecular mechanisms for self-renewal, maintenance of pluripotency and lineage specification are poorly understood. However, emerging data point to a key role for epigenetic mechanisms, including nuclear architecture, chromatin structure, chromatin dynamics and histone modifications, in these fundamental processes. Here, we review recent findings on the structure and function of chromatin and nuclear architecture in ES cells and discuss models for the maintenance of pluripotency, self-renewal and lineage specification.

Nuclear architecture

The nucleus of eukaryotic cells is now recognized as a well-organized structure, and the architectural integrity of the nucleus is important for faithful genome function[3,4]. The mammalian nucleus contains a large number of distinct subcompartments, which consist of unique sets of resident proteins and in which specific functions, such as transcription and RNA processing, are carried out. In addition, genomes are non-randomly organized within the cell nucleus[5]. Chromosomes occupy discrete chromosome territories[6], and single genes are arranged in non-random positioning patterns within both the chromosome territory and the nucleus[5]. These preferential positions are probably functionally important as they vary among tissues, during development and among cell types[7–9].

> “The molecular mechanisms for self-renewal, maintenance of pluripotency and lineage specification are poorly understood. However, emerging data point to a key role for epigenetic mechanisms ...”

Only a few aspects of nuclear architecture have been characterized in detail in ES cells, but cursory observations indicate that many nuclear features, including the nuclear lamina[10], the nucleolus, heterochromatin structure[11,12] and nuclear speckles (nuclear domains that are enriched in pre-mRNA splicing factors), undergo morphological changes during the differentiation process (FIG. 1). The absence of lamin A, one of the main nuclear structural proteins, even serves as a specific stem-cell marker in both mouse and human ES cells[10]. Other nuclear features seem to be largely unaltered. For example, comparable distributions of centromeres and of promyelocytic leukaemia (PML) bodies (which are implicated in transcription, apoptosis and cellular stress processes) are found in both human ES cells and differentiated cells[13]. A complete characterization of the nuclear landscape in pluripotent ES cells will be a useful basis for a cell-biological understanding of these cells.

The spatial positioning of chromosomes and genes has been related to their functional activity, and functionally related changes in global genome organization have been documented in numerous differentiation systems and in development[14,15]. However, initial studies indicate that the large-scale organization of genomes at the level of chromosomes is not significantly different in human ES cells compared to differentiated cells[13]. Chromosomes 18 and 19 and chromosome 6, which contains the pluripotency marker *OCT4*, are in similar positions in human ES cells and differentiated cells[13], which indicates that chromosome-positioning patterns are already established at this time. This finding is intriguing as repositioning of chromosomes has been reported during differentiation of several lineage-committed cell types and among different tissues[7,16]. More in line with those observations, the position of at least some chromosomes might be variable, as human chromosome 12, which contains a genomic region that is enriched with ES-cell-specific genes[17], including the stem-cell marker *NANOG*, changes its preferential nuclear position and localizes significantly more centrally in human ES cells compared with B cells[13]. This indicates that the global level of activity of this genome region might contribute to its location within the cell nucleus.

The positioning of chromosomes within the nucleus might not be of functional relevance in ES cells, or in any other cell type for that matter; the spatial organization of single genes, however, might be because several key ES-cell genes undergo significant

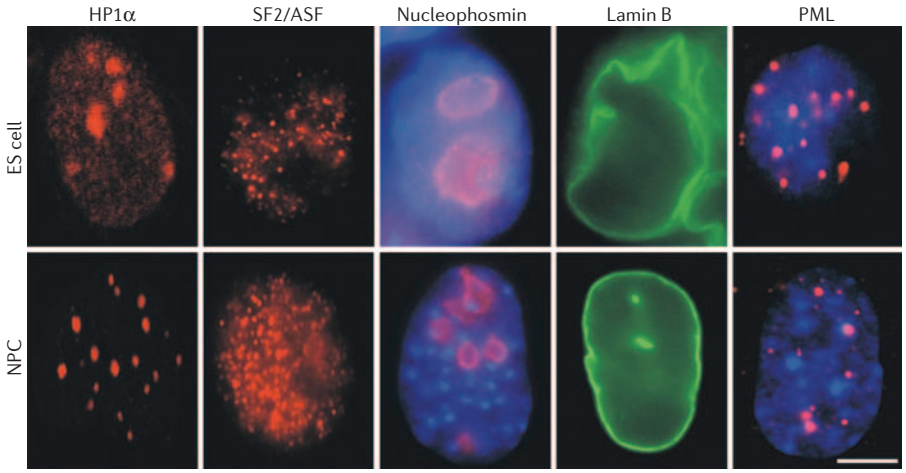


Figure 1 | **Nuclear architecture in ES cells and differentiating ES-derived cells.** Nuclear domains in an undifferentiated embryonic stem (ES) cell (top) and a differentiating ES-derived neuronal progenitor cell (NPC, bottom). From left to right: heterochromatin, as detected with an anti-HP1α antibody, is confined to fewer and larger foci in ES cells compared with NPCs; nuclear speckles, as detected with an anti-SF2/ASF antibody, appear as small, dispersed foci in ES cells and become more conspicuous in NPCs; nucleoli, as identified with an anti-nucleophosmin antibody, appear larger in ES cells compared with NPCs; the ill-defined nuclear lamina in ES cells, stained with an anti-lamin B antibody, becomes round and distinct in NPCs; promyelocytic leukaemia (PML) bodies labelled with an anti-PML antibody show similar patterns in ES cells and NPCs. DAPI, blue. Scale bar, 5 μm.

positional changes during differentiation that are similar to those observed in other differentiation systems. *NANOG* relocates from a more peripheral position in human ES cells compared with B cells, and the *OCT4* gene seems to be looping out from its chromosome territory[13]. A similar chromosomal extrusion was observed for the *HoxB* locus in mouse ES cells after differentiation was induced with retinoic acid (RA)[18]. Whether these positional changes are a cause or consequence of the transcriptional status and whether they are required for proper regulation is unknown.

The notion that global genome organization is established early and is stable, but that local organization is more plastic and might significantly contribute to genome regulation, is also supported by observations of replication timing. Whereas active genes generally replicate early in S phase, silenced genes usually replicate late, and replication timing patterns generally provide a good representation of the global genome organization[19]. Indeed, global replication patterns are essentially identical between pluripotent murine ES cells and several types of differentiated cell[20]. However, the replication timing of several stem-cell and lineage-specific genes correlates with their activity. Whereas ES-cell-specific genes, including *NANOG*, delayed their time of replication after neural induction, which is consistent with their inactivation[21], several neuronal genes, including *SOX3* and *MASH1*, replicated early, which accompanied their activation[21,22]. Surprisingly, most of the analysed genes, including lineage-restricted genes such as *IKAROS*, *MYOG* and *MATH1*, replicated early in S phase, possibly indicating that these genes are primed for activity[21,23]. Taken together, these observations indicate that the global genome organization is established early in ES cells, but that, at the same time, the local genome organization remains plastic.

> "ES cells seem to be characterized by a distinct higher-order global chromatin structure ..."

Chromatin structure and dynamics

The spatial organization of chromatin into higher-order structure has recently emerged as a key contributor to genome regulation[24]. Chromatin structure can influence gene function by affecting the accessibility of regulatory proteins to their target site and by modulating the affinity of transcriptional regulators with their targets. ES cells seem to be characterized by a distinct higher-order global chromatin structure (FIG. 2).

Studies in several systems indicate that ES cells are richer in less compact euchromatin and, as differentiation progresses, accumulate highly condensed, transcriptionally inactive heterochromatin regions[3,25]. As a crude measure for these changes, in embryonal teratocarcinoma F9 stem cells[26], as well as in murine ES cells[12], heterochromatin spatially rearranges and the number of heterochromatin foci increases during differentiation (FIG. 1). Transmission electron-microscopy studies reveal a transition from fine granular chromatin in undifferentiated human ES cells to irregularly shaped heterochromatic nuclei in RA-induced differentiating cells[11]. Direct visualization of centromeric heterochromatin with a probe against the major satellite-repeat sequence reveals a more diffuse heterochromatin structure in undifferentiated ES cells versus more compact heterochromatin with well-defined foci in ES-derived neuronal progenitor cells (NPCs)[12].

Consistent with this notion, the expression of several ATP-dependent chromatin-remodelling factors is elevated in ES cells[27] and the genomic disruption of chromatin-remodelling proteins, including BRG1 (REF. 28), SNF5 (REF. 29), SSRP1 (REF. 30) and SNF2H[31], results in premature embryonic death prior to implantation. Interestingly, in all these cases, lethality occurred at the blastocyst stage, when the inner cell mass (ICM), the source of all ES cells, is formed. In *Drosophila melanogaster*, chromatin remodelling is also involved in germline stem-cell self-renewal and differentiation, as indicated by the failure of an ATP-dependent chromatin remodelling factor ISWI mutant to differentiate, and by the emergence of defects in self-renewal in mutants of the chromatin remodeller DOM[32]. Furthermore, in mouse ES cells, the chromatin remodelling NuRD (nucleosome remodelling and histone deacetylation) complex is essential for differentiation[33]. Although the detailed mechanism of their functions is unknown, these observations strongly point towards an active role of chromatin-remodelling factors in the maintenance of stem-cell identity and the initial steps of differentiation.

The chromatin organization within the nucleus is largely maintained by the binding of structural chromatin proteins. Most prominent among those are the core and linker histones, which form the proteinaceous structural backbone of chromatin (BOX 1). Whereas core histones are stably bound to chromatin with a residence time of several hours, most other DNA-binding proteins undergo remarkably transient interactions with native chromatin *in vivo* with residence times that range from a few minutes for linker histones to a few seconds for most transcription factors, remodellers and even structural proteins, including

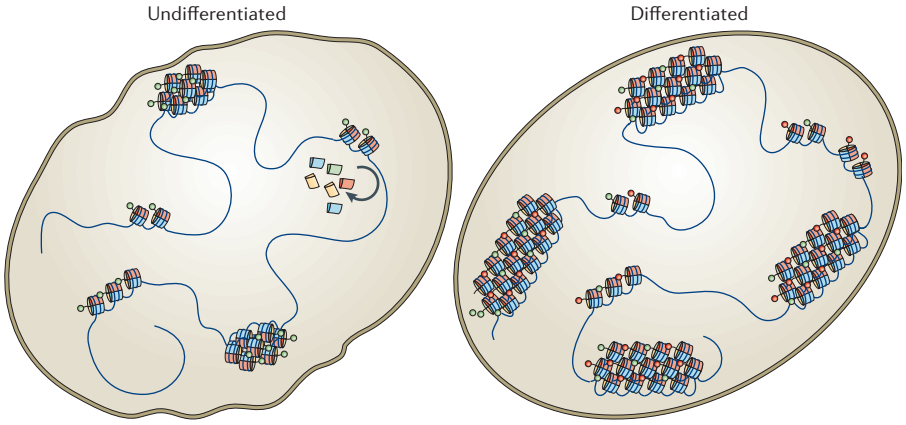


Figure 2 | **Chromatin during ES-cell differentiation.** In pluripotent embryonic stem (ES) cells (left), chromatin is globally decondensed, enriched in active histone marks (green circular tags), and contains a fraction of loosely bound architectural chromatin proteins. As cells differentiate (right), regions of condensed heterochromatin form, silencing histone marks (red circular tags) accumulate, and structural chromatin proteins become more stably associated with chromatin.

heterochromatin protein HP1 and the high mobility group (HMG) proteins[34,35].

Although the functional relevance of these dynamic properties has not been entirely clear, recent observations in pluripotent ES cells indicate that the dynamic interplay of proteins at chromatin is a crucial component in genome function[12]. *In vivo* dynamic measurements and biochemical analysis indicate that pluripotent ES cells contain a population of architectural proteins that is only loosely associated with chromatin, including core and linker histones, as well as the heterochromatin protein HP1 (REF. 12) (FIG. 2). This hyperdynamic population represents typically 10–25% of the total protein population and is characterized by residence times of a few seconds. The presence of this population is a true hallmark of pluripotent cells and not merely of undifferentiated cells, as several undifferentiated but already lineage-committed cell lines, such as C2C12 myoblasts or PC12 rat pheochromocytoma cells, do not contain it[12]. Although it is not clear at present whether the altered binding properties of this subfraction of architectural chromatin proteins is due to post-translational modifications or is a consequence of the global changes in chromatin structure, this loosely bound fraction seems to be functionally important as interference with this population affects the differentiation kinetics of ES cells[12].

One possibility is that this pool of architectural chromatin proteins provides essential building blocks for the formation of chromatin domains as cells implement their specific expression programmes by the targeted sequestration of genes into active and repressive chromatin domains. In this scenario, the loosely bound core histones and architectural chromatin proteins might be recruited to genome regions that are destined for silencing as cells begin to differentiate. Chromatin-assembly factors and remodellers would facilitate their incorporation at these sites and would attract histone- and DNA-modifying enzymes to mark these regions epigenetically. Large regions of silenced chromatin will probably undergo changes in higher-order chromatin structure and form the abundant heterochromatic regions that are observed as cells enter differentiation. At the same time, the dynamic nature of chromatin-associated proteins might also make it easier for regulatory factors to gain access to regulatory sequences, and to rapidly activate complex lineage-specific gene-expression programmes.

Box 1 | **Histones and histone variants**

Coined around 1880 by W. Flemming, the term 'chromatin' refers to the structure of chromosomes during interphase. Chromatin is composed of DNA that is wrapped around core 'histones' to form the now-accepted 'beads on a string' model, according to which 147 bp of DNA occupy one nucleosome octamer unit that consists of two pairs each of H2A–H2B and H3–H4 core histone dimers. Core histones are predominantly assembled onto chromatin during S phase, through the actions of histone chaperones. Core histones therefore provide the structural backbone for chromatin structure and are largely regulated by post-translational covalent histone modifications such as methylation, acetylation, phosphorylation, ubiquitylation and sumoylation. Because the N-terminal tails of the core histones protrude outside the nucleosome structure, tail modifications are regarded as key determinants of chromatin structure. Although there are exceptions, acetylation is normally associated with the active euchromatin, whereas methylation usually marks the inactive heterochromatin. Chromatin structure can also be modified by ATP-dependent chromatin-remodelling factors, which use energy to transiently alter the chromatin conformation and facilitate accessibility for the transcriptional apparatus. In addition to the four core histones, several replacement histone variants for either H2A or H3 exist. The H2A variants include H2A.Z, H2A.X, macroH2A and H2A.Bdb, and the H3 variants comprise H3.1, H3.2, H3.3 and CenpA. Unlike core histones, the histone variants are transcribed from a polyadenylated mRNA and are assembled into chromatin independently of DNA replication. Histone variants reflect an additional means by which a cell can regulate chromatin structure and function.

Chromatin modifications

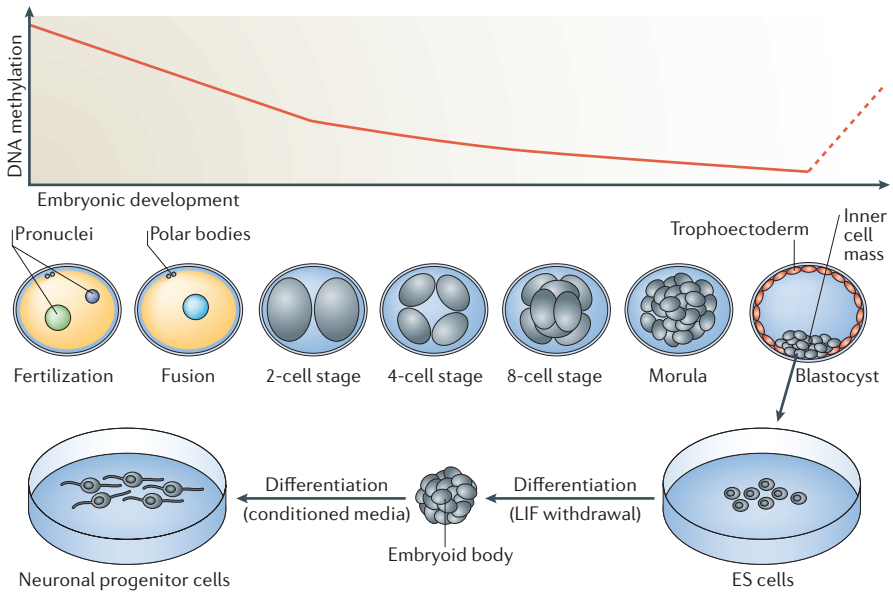
Post-translational modifications of core histones, as well as the methylation of genomic DNA, have been correlated with chromatin states and the transcriptional status of genes[36,37]. Consistent with changes in the global genome activity, changes in histone-modification patterns accompany ES-cell differentiation[38]. Examples are the differentiation-dependent increase in the silenced chromatin mark tri-methylated residue K9 of histone H3 (H3-triMeK9) and a decrease in the global levels of acetylated histones H3 and H4 (REFS 12,38,39), which is usually associated with active chromatin regions. These observations indicate that ES-cell chromatin is overall more active, or at least marked with activity-associated histone modifications, and that differentiation is accompanied by a transition to transcriptionally less-permissive chromatin.

An elevation of repressed heterochromatin marks, including H3-triMeK9, H3-MeK27, H3-diMeK27, H4-diMeK20 and H4-triMeK20, was also observed in repeat sequences and retrotransposons during RA-induced mouse ES-cell differentiation[40]. In agreement with the predominance of activating marks in ES-cell chromatin, nuclear fusion of somatic T cells with murine ES cells, which renders the T cells pluripotent, resulted in global H3 and H4 hyperacetylation as well as global hypermethylation of H3–K4 of the somatic genome[41]. The functional relevance for global histone deacetylation during ES-cell differentiation is implied by the observed inhibition of differentiation of murine ES cells after treatment with the histone deacetylase (HDAC) inhibitor trichostatin A (TSA)[38].

As well as global changes, local histone modifications are thought to be important for the proper control of differentiation-specific genes. As would be expected, the promoter of the ES-cell marker *OCT4* is enriched for the active mark H3-triMeK4 in undifferentiated, but not in differentiating, ES cells[38]. A particularly intriguing temporal pattern of histone modifications occurs in the lineage-specific λ5-*VpreB1* locus, which is expressed during B-cell differentiation, but not in ES cells[42]. Although the locus is inactive, it is already marked for activity in undifferentiated ES cells by histone H3 acetylation and histone H3-K4 methylation. When ES cells were differentiated into a non-lymphoid lineage, these active marks were removed from this locus[42], which indicates that the maintenance of transcriptionally competent chromatin is an active process that is maintained by histone modifications that help to preserve the pluripotent state. Similar findings were shown for the neuronal gene *NFM*, which contains active chromatin marks in undifferentiated ES cells, despite its transcriptional inactivity[41]. Importantly, whereas T-cell *NFM* chromatin was unmodified, the *NFM* locus in reprogrammed hybrid thymocytes that were fused with ES cells showed similar histone-modification patterns to those of undifferentiated ES cells[41]. An interesting possibility is that these loci are marked for transcriptional competence and for expression later in the differentiation process[43].

Correlations between ES-cell differentiation status and DNA methylation, which is a general indicator of silenced genome regions[44], have also been made. DNA methylation has a direct role in regulating chromatin structure[45], and is essential for the establishment of chromatin structure during development[46]. During early embryogenesis, global methylation patterns are erased and a wave of *de novo* DNA methylation follows after implantation[47] (BOX 2). Global surveys of CpG methylation islands in ES cells and in ES-cell-derived embryoid bodies revealed both specific methylation and de-methylation patterns during ES-cell differentiation[48]; global methylation, however, seems to be a functionally relevant landmark, as the treatment of partially differentiated ES cells with the demethylating agent 5-azacytidine (5-AzaC) induces de-differentiation[49]. In other cell systems, such as C3H/10T1/2 cells, 5-AzaC induced differentiation into striated muscle cells, adipocytes and chondrocytes[50]. Although this might seem a contradiction, the authors speculate that 5-AzaC causes a reversion of C3H/10T1/2 cells to a more pluripotent state, from which the generated lineages subsequently arise[50]. Supporting this view, the treatment of trophoblast stem cells — which do not express *Oct4* and do not contribute to the embryo proper — with 5-AzaC, caused the activation of the *Oct4* gene in these cells[51]. These data support a role for global DNA methylation during stem-cell differentiation.

Box 2 | **DNA methylation during early embryonic development**



Immediately following fertilization, both the maternal and paternal genomes undergo rapid global DNA demethylation. The first active phase of demethylation occurs exclusively on the paternal genome before the first round of replication[62]. This is followed by a passive demethylation phase on both genomes[63], during which global DNA methylation is diluted after every round of DNA replication. Demethylation reaches a nadir at the blastocyst stage (day 3.5 in the mouse; see figure), when most of the inherited methylation marks — with the exception of imprinted genes — have been erased. Methylation resumes following implantation. The embryonic ectoderm and mesoderm become hypermethylated through an active *de novo* methylation process, whereas the primitive endoderm and trophoblast remain hypomethylated. As embryonic stem (ES) cells are extracted from the inner cell mass at the blastocyst stage, they are hypomethylated when first cultured, but gradually acquire methylation marks during prolonged passaging[64]. Cultured ES cells can mimic the initial stages of embryonic development by turning into ball-shaped embryoid bodies when leukaemia inhibitory factor (LIF), which is required to maintain pluripotency, is withdrawn from the culture media. Embryoid bodies can be replated in conditioned medium to allow differentiation into multiple cell types.

The transcriptional landscape in ES cells

From what we have learned about chromatin in pluripotent ES cells, it is clear that many properties of chromatin are distinct compared to differentiated cells. In particular, it seems that ES-cell chromatin has numerous hallmarks of highly active chromatin, including altered higher-order structure, an accumulation of activating histone modifications, an abundance of chromatin-remodelling factors, a reduction of DNA methylation and a hyperdynamic interaction with chromatin proteins. So how, if at all, does altered chromatin architecture contribute to the unique properties of stem cells, including pluripotency and their potential to self-renew, and how does the chromatin status in ES cells affect the specification of lineage-specific genome-expression programmes? There are a number of fundamentally distinct models that can be considered.

On the one hand, as ES cells are not committed to any particular function, a possible view is that these cells express, in addition to their housekeeping genes, a minimal set of genes that are responsible for self-renewal and maintenance of pluripotency (FIG. 3a). Following differentiation, these 'stemness' genes (that is, genes that are specific for stem cells) are silenced and expression of lineage-specific transcription factors triggers sets of lineage-specific genes. This hierarchical activation (HA) model is consistent with genetic transcriptional networks that operate during differentiation and development[43].

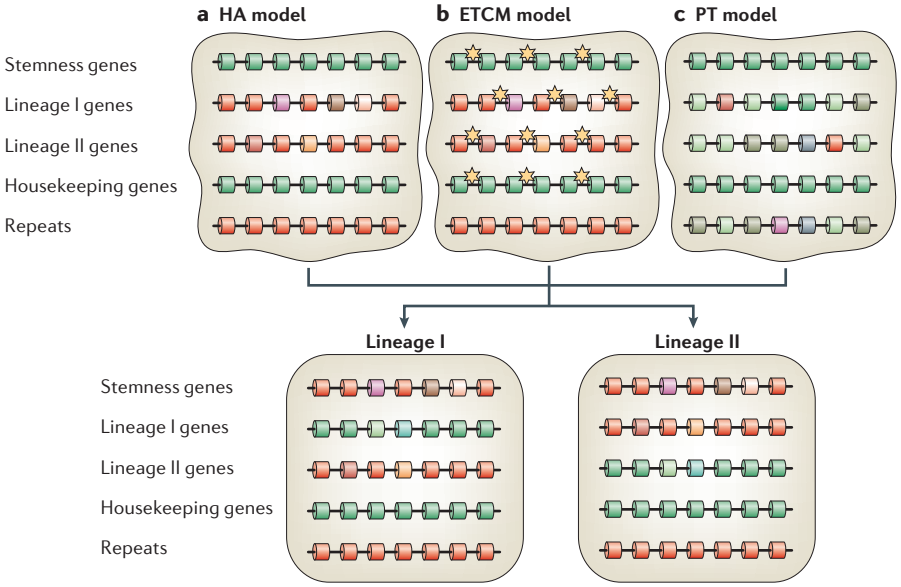


Figure 3 | **Models of the transcriptional landscape during ES-cell differentiation. a** | Hierarchical activation (HA) model. Only housekeeping genes and genes that are necessary for the maintenance of pluripotency and self-renewal are active (green). During differentiation, these 'stemness' genes are turned off, whereas lineage-specific genes are selectively turned on. **b** | Early transcription competence marks (ETCM) model. Although only housekeeping and stemness genes are active, lineage-specific genes are epigenetically marked for activity (stars). **c** | Promiscuous transcription (PT) model. In addition to the highly expressed housekeeping and stemness genes, chromatin in embryonic stem (ES) cells supports genome-wide transcription at low levels of lineage-specific genes as well as repeats. During differentiation, silencing of chromatin domains and the formation of heterochromatin prevents this promiscuous transcription and the activity becomes limited to lineage-specific genes.

A second model envisions that tissue-specific genes are not active in ES cells, but are epigenetically marked for expression at a later stage (FIG. 3b). According to this model, active chromatin modifications are abundant in ES cells, but these marked regions are not necessarily active but are merely primed. The selection of these genome regions would occur by the association of sequence-specific factors, which subsequently recruit histone-modifying activities to lineage-specific genes that are destined for late expression[43]. This early transcription competence marks (ETCM) model is based on the observation that several lineage-specific genes, which are repressed in ES cells, nevertheless contain active chromatin marks[23,42,43,52].

An alternative model is the promiscuous transcription (PT) model (FIG. 3c). In this view, ES cells express a set of stem-cell-specific genes that are controlled by stem-cell-specific transcription factors; but, in contrast to a strictly hierarchical model, the rest of the genome is not completely silenced, but most genome regions are expressed indiscriminately at low levels. As cells differentiate, a global reduction of gene activity occurs, including silencing of the stemness genes, and at the same time sets of lineage-specific genes are selected to be maintained in an active status. It is probable that their activity is reinforced by increased expression of lineage-specific transcription factors. In this model, lineage specification would occur through stochastic clonal selection from a repertoire of stem cells that express large fractions of the genome. It is possible that clonal selection is not entirely random, as there is evidence that ES cells enter the neuronal lineage by default[53].

Current data do not allow us to distinguish between the various models. The ETCM and PT models are supported by the presence of fewer heterochromatic regions and the more dynamic interaction of architectural proteins with chromatin. In contrast to the HA model and the ETCM model, in which only a subfraction of the genome would be expected to be active in ES cells, according to the PT model large segments are expected to be transcriptionally active (FIG. 3c). In support of this, transcriptional profiling revealed that more genes are expressed in undifferentiated ES cells than in other cell types[54,55], including haematopoietic or keratinocytic cells[56]. Furthermore, a survey of the core transcriptional regulatory circuits in human ES cells indicates that the three 'classic' ES-cell transcription factors *OCT4*, *NANOG* and *SOX2* occupy a surprisingly large number of gene promoters in human ES cells[57]. Interestingly, almost half of those were transcriptionally inactive genes, which indicates that these transcription factors are involved both in activation and repression[57].

The PT model is further supported by the observation that RNA from normally silenced major and minor satellite repeats and centromeric repeats is present in ES cells, and additional repeat sequences, transposons and retrotransposons are similarly active[40,58]. Moreover, although not surveyed specifically in cultured ES cells, a higher activity of transposable elements within the genome is evident in early pre-implantation mouse embryos[59]. Furthermore, the transcription-associated histone H3.3 is the only core histone that is not present in a more dynamically bound fraction in ES cells, which is consistent with a larger fraction of transcriptionally active genome regions in ES cells[12].

The putative low levels of global transcription in the PT model might have no specific function and be simply a by-product of the altered chromatin structure. On the other hand, it is tempting to speculate that the low-level expression, particularly of non-coding regions, might be essential in the differentiation process. Considering that heterochromatin formation is now known to involve the RNA interference (RNAi) machinery, as deletion of RNAi machinery components results in the aberrant accumulation of centromeric heterochromatin transcripts[60], an intriguing possibility is that the expression of repeats is required for the formation of heterochromatin blocks during the differentiation process. The relevance of this mechanism is indicated by the observation that differentiation is perturbed in ES cells that lack Dicer, the enzyme that is responsible for the cleavage of double-stranded RNAs into small interfering (si)RNAs[61].

Each of the models makes distinct, testable predictions. In a minimal HA model, the transcriptional profile is expected to be highly punctuated by a relatively minor fraction of highly active, stem-cell-specific genes. The background would largely be provided by housekeeping genes, and lineage-specific genes are predicted to be completely silenced. By contrast, in a PT model, large proportions of the genome are predicted to be transcribed, including low levels of lineage-specific gene transcripts and intragenic transcripts, and transcription that originates from repeats. The ETCM model can be tested by the genome-wide analysis of histone modifications, which are expected to cluster in tissue-specific genes

in pluripotent ES cells[43]. Distinguishing between these possibilities will be a milestone in our understanding of stem cells.

Concluding remarks

ES cells are still enigmatic and our knowledge of what makes a stem cell is rudimentary. In all likelihood, chromatin holds some of the keys to understanding ES cells and unlocking their full potential. The next step will be to describe in detail the factors that contribute to the maintenance of the pluripotent ES-cell chromatin, particularly the role of chromatin-remodelling proteins, chromatin-associated proteins and histone-modifying proteins. In parallel, it will be essential to describe the transcriptional landscape of ES cells during the differentiation process, and to map on a genome-wide scale the histone modifications during this process. Despite our limited current knowledge, there is little doubt that we will eventually uncover the molecular mechanisms that give rise to the unique properties of ES cells and that we will then be able to exploit them as powerful tools in basic discovery and clinical applications. Understanding chromatin in ES cells will be instrumental in this endeavour.

Note added in proof

During preparation of this article, a series of reports have implicated polycomb-group proteins (PcG) in the maintenance of the pluripotent state. PcG proteins act in a repressive fashion by binding to H3-triMeK27, a repressive epigenetic mark. These studies have mapped the localization of PcG proteins throughout the genome and find PcG complexes binding to more than 1,000 genes, many of which are differentiation specific[65–67]. Along the same lines, Azuara *et al.*[23] and Bernstein *et al.*[52] demonstrated the presence of H3-triMeK27 on repressed lineage-specific genes in undifferentiated ES cells. However, intriguingly, they detected the simultaneous presence of markers of open chromatin, such as H3-MeK4. These observations indicate that lineage-specific genes are poised for activity but are held in check by the repressive machinery, and they point to a model in which the state of ES cells is determined by the modulation of a dynamic equilibrium between gene activity and repression.

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doi:10.1038/nrm1938
Published online 17 May 2006

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Acknowledgements
We thank M. Bustin, N. Dillon, P. Scaffidi and T. Takizawa for constructive comments. T.M. is a Fellow of the Keith R. Porter Endowment for Cell Biology.

Competing interests statement
The authors declare no competing financial interests.

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