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Chromatin plasticity and genome organization in pluripotent embryonic stem cells

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In search of the mechanisms that govern pluripotency and embryonic stem cell (ESC) self-renewal, a growing list of evidence highlights chromatin as a leading factor, controlling ESC maintenance and differentiation. In-depth investigation of chromatin in ESCs revealed distinct features, including DNA methylation, histone modifications, chromatin protein composition and nuclear architecture. Here we review recent literature describing different aspects of chromatin and genome organization in ESCs. The emerging theme seems to support a mechanism maintaining chromatin plasticity in ESCs but without any dramatic changes in the organization and nuclear positioning of chromosomes and gene loci themselves. Plasticity thus seems to be supported more by different mechanisms maintaining an open chromatin state and less by regulating the location of genomic regions.

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Introduction

Embryonic stem cells (ESCs), which are derived from the inner cell mass (ICM) of the mammalian blastocyst before implantation in the uterus, are able to self-renew indefinitely and to differentiate into all cell types of the three germ layers. This dual capacity makes ESCs an excellent model system for studying development and a potential source for cell and tissue replacement. Hopes for regenerative medicine considerably increased recently, with the discovery that somatic, differentiated cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by the introduction of only a few transcription factors [1,2]. Notably, direct reprogramming of patient cells into iPSCs overcomes the immunogenic limitations associated with embryo-derived cells and perhaps more importantly, eliminates the ethical concerns involving the use of human embryos for the derivation of human ESC (hESC) lines. However, in order to make these potential clinical applications a reality, intensive research on the mechanisms of pluripotency and ESC differentiation must be carried out. The regulatory transcriptional network of pluripotency factors and the epigenetic state of ESCs are most rigorously pursued (for reviews see [3–6]). However, study of chromatin structure, dynamics and organization is also central to the understanding of the maintenance of self-renewal and pluripotency, with ESCs serving as a gold standard [7–9].

In this review, we describe recent findings on chromatin and genome organization in ESCs and discuss how it yields the unique transcriptional profile found in ESCs, and why we believe it contributes to maintaining the pluripotent state.

Chromatin organization in pluripotent stem cells

The chromatin of pluripotent stem cells is believed to have unique characteristics, including an open conformation and a hyperdynamic association of chromatin proteins, reflecting the plasticity of the genome in pluripotent cells [7] and likely contributing to the maintenance of pluripotency and self-renewal [10,11•].

The association between chromatin and its structural proteins, including HP1α, H1 and core histones, was found to be less rigid in pluripotent cells, as demonstrated both by biochemical salt extractions [11•] and by fluorescence recovery after photobleaching (FRAP) [11•,12•] (Figure 1a). Curiously, not all chromatin proteins behave this way. The histone variant H3.3 and the linker histone H1.5 bind tightly in both pluripotent and committed cells [11•,12•], implying that the dynamic association of chromatin proteins with chromatin in ESCs is a regulated process and may not simply reflect a decondensed chromatin structure which renders all chromatin proteins hyperdynamic.

Heterochromatin rearrangement during embryonic stem cell differentiation

Consistent with a global open chromatin state in ESCs, quantitative electron microscopy showed that heterochromatin foci are more frequent in the differentiated neuronal progenitor cells (NPCs) than in ESCs [13••], suggesting that heterochromatin formation and maturation occurs during ESC differentiation.

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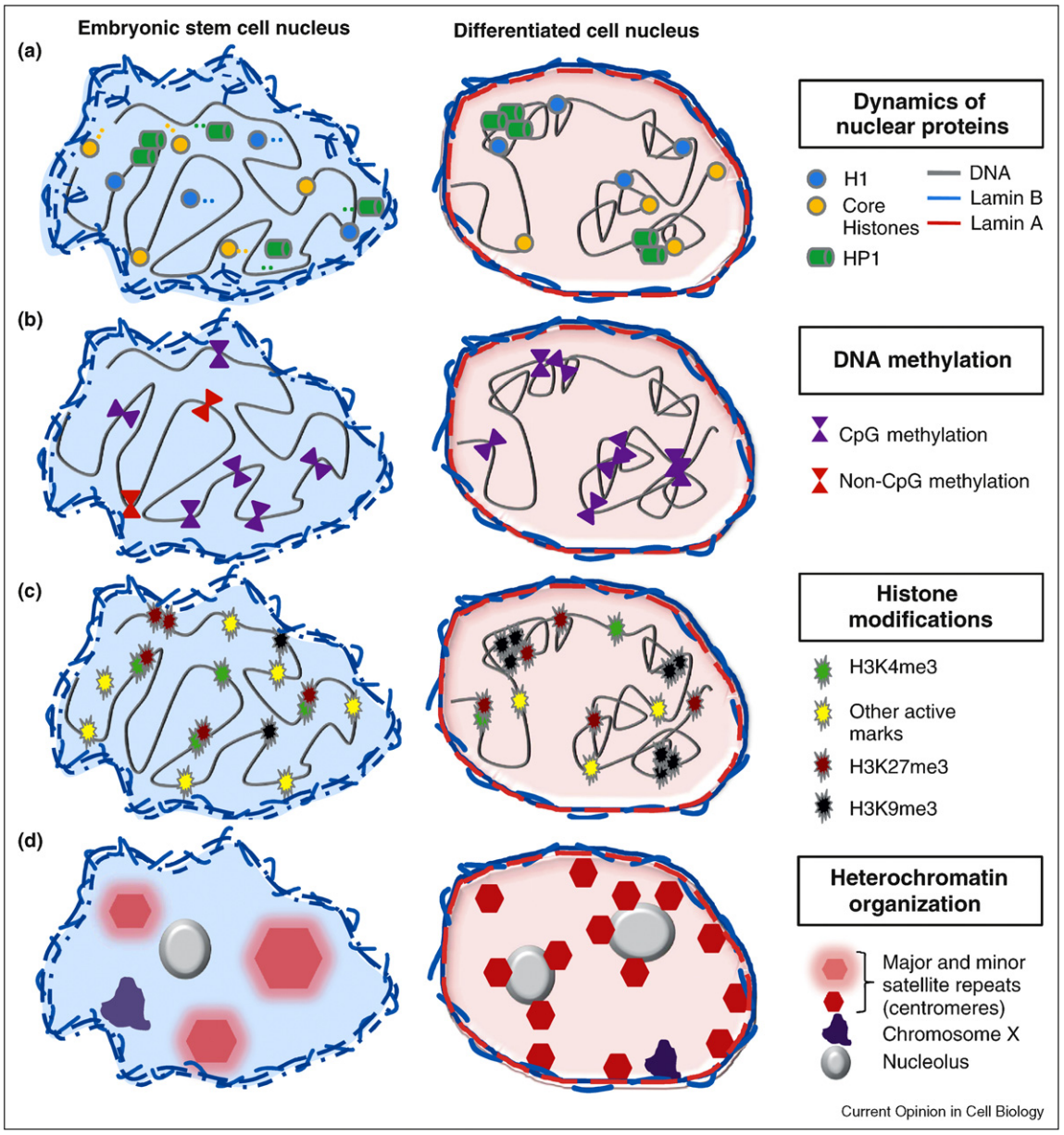
Figure 1



A schematic view of chromatin and genome characteristics in ESCs and in early differentiation. **(a)** Chromatin protein dynamics. Chromatin proteins such as the linker histone H1 and core histones are more dynamically associated with chromatin in ESCs than in differentiated cells. HP1α and lamin B nuclear proteins also bind more loosely in pluripotent cells [11•,12•]. Lamin A expression and localization in the nuclear lamina occur during early differentiation [36]. **(b)** DNA methylation. In mammalian somatic cells, DNA methylation is present on cytosines in a CpG context. In pluripotent ESCs, 25% of the cytosine methylation sites in the genome are found in a non-CpG context, suggesting that ESCs utilize a unique DNA methylation program [25••]. **(c)** Histone modifications. The global levels of several histone modifications differ between ESCs and differentiated cells. This includes several active marks which are more abundant in ESCs [11•,13••,43,47], and repressive marks which are enriched in differentiated cells, and which accumulate in well-defined foci (H3K9me3)[11•,35,45•]. Bivalent marks (H3K4me3 together with H3K27me3) are found on promoters of developmentally regulated genes in ESCs, some of which resolve into a single modification in differentiated cells [27,28,31]. **(d)** Centromeric heterochromatin. Major and minor satellite DNA repeats which are normally found at the heterochromatic centric and pericentric regions, are dispersed in ESCs, but form dense foci upon differentiation [11•,17]. Accordingly, centromeres are redistributed next to nucleoli and the nuclear periphery in differentiated cells [45•]. The inactive X chromosome in female somatic cells which is represented in the scheme is also repositioned next to the nuclear envelope [72]. Telomeric chromatin is apparently not displaced in the nucleus, but in ESCs it has a more open structure [19•].

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A specific feature of heterochromatin in ESCs is the nuclear distribution of HP1α, β and λ proteins. HP1 distribution was found to be diffuse in hESC nuclei in contrast to the distinct foci observed in nuclei of differentiated cells, in which HP1 variants mostly associate with pericentric heterochromatin [14,15]. However, in mouse ESCs (mESCs) this diffuse pattern was not observed: HP1 variants form foci in E14 ESCs and the number of these heterochromatin-associated foci increase during differentiation [16]. These observations indicate that some differences in heterochromatin organization may be present between mouse and human ESCs.

Two of the main residents of constitutive heterochromatin are the major and minor satellite repeat sequences, which are commonly methylated and located in centromeric regions of the genome. Major satellite repeats seem to localize diffusely in ESC nuclei and form well-defined chromocenters in NPCs [11•]. Direct visualization of methylated heterochromatin using the methyl-DNA binding protein MBD fused with GFP revealed an increase in the number of MBD foci during ESC differentiation [17], suggesting a global redistribution of heterochromatin during ESC differentiation. Interestingly, major and minor satellite repeats, as well as other repetitive sequences, which are normally repressed in differentiated cells, are highly transcribed in mouse ESCs [13••,18], and become associated with heterochromatin-related histone modifications after differentiation [18,19•]. Along the same lines, telomeric chromatin is associated with increasing levels of H3K9me3 and H4K20me3 and reduced sensitivity to micrococcal nuclease (MNase) digestion during ESC differentiation [19•]. Incorporation of the histone variant H3.3 — which is associated with transcriptionally active chromatin — at interphase telomeres in ESCs, also suggests that telomeric chromatin has special features and may be less condensed and repressed in pluripotent cells [19•,20]. Accordingly, telomeric transgenes are expressed at low levels in ESCs but not at all in differentiated cell types [21]. These studies indicate that chromatin organization is profoundly different in ESCs, and that this apparent open configuration may lead to transcriptional activity of heterochromatin domains.

Epigenetic mechanisms

DNA methylation is an important epigenetic mechanism for the regulation of gene expression during mammalian development. Promoters of transcription factors involved in pluripotency are generally methylated in differentiated cells but hypomethylated in ESCs and iPSCs [22]. Indeed, reprogramming involves demethylation of pluripotency-related promoters in a process that seems to be both replication-independent and AID-mediated [23]. But the global levels of DNA methylation covering most CpG islands are not substantially different between ESCs and differentiated cells [24]. Intriguingly, a recent single nucleotide survey in hESCs showed that 25% of DNA methylation regions are not in a CpG context and are demethylated upon differentiation, implying that ESC DNA is subjected to different methylation mechanisms [25••] (Figure 1b). It would be interesting to test if the newly discovered DNMT3BΔ5 splice form, which is highly expressed in ESCs [26], is involved in the regulation of this unique methylation pattern.

An additional, more explored level of epigenetic regulation in ESCs is post-translational modifications of histones. Histone modifications dictate chromatin structure and transcriptional competence of essentially all eukaryotic genes. Histone H3 tri-methylated on lysine 4 (H3K4me3), for example, is associated with transcriptionally active chromatin whereas the polycomb group-associated H3K27me3 modification mark is associated with suppressed facultative heterochromatin. Curiously, H3K4me3 and H3K27me3 are often found on the same promoters, forming a 'bivalent' mark. In ESCs, this bivalent mark seems to operate on key developmental genes [27,28], keeping genes poised for activation, likely by associating with a transcriptional-competent (phosphorylated on serine 5) form of RNAPII [29–31]. Nevertheless, bivalent marks are not unique to ESCs and are found in different cell types and during various stages of development [32,33].

Intriguingly, one of the two bivalent modifications, H3K27me3 is enriched at the nuclear periphery in mESCs, and although the levels of H3K27me3 seem to remain unchanged during mESC differentiation, the perinuclear localization decreases [34•,35]. The nuclear lamina, found at the nuclear periphery of ESCs comprises mostly of lamin B proteins, which are more dynamic in ESCs than in other cell types [12•] (Figure 1a). Additionally, undifferentiated ESCs lack lamin A/C proteins [36], which only appear during early ESC differentiation (Figure 1a). In human fibroblasts, lamin B associates with chromatin domains which are mostly silenced and highly enriched with H3K27me3 [37]. Finally, polycomb target genes, many of which are developmental regulators, are repressed in ESCs and carry the H3K27me3 modification [38,39]. Therefore, it is tempting to speculate that polycomb target genes are located in proximity to the nuclear envelope, serving as a specific repressive mechanism in ESCs (Figure 2). Moreover, since ESCs seem to mostly utilize facultative heterochromatin-mediated repression (e.g. by H3K27me3) rather than constitutive heterochromatin-mediated repression (e.g. by H3K9me3), the transcriptional silencing may not be as firm as in differentiated cells, resulting in leaky, permissive transcription [40,41].

Supporting this notion is the relative enrichment of activity-associated histone modifications in ESCs (Figure 1c). Histone acetylation, for example, is usually associated with transcriptionally active chromatin. ESCs, which maintain a transcriptional-competent program [13••,42], possess high levels of histone H3 and H4

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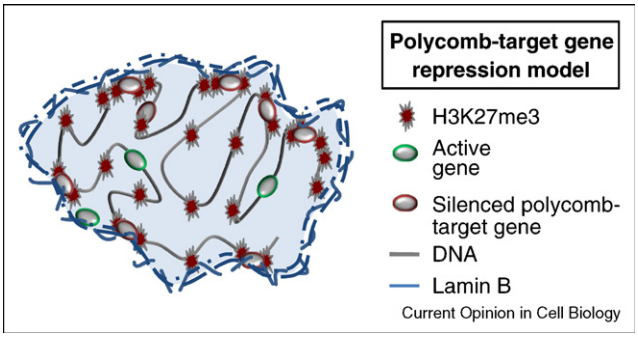
Figure 2



Polycomb target gene repression model. The repressive component of the bivalent modification H3K27me3, has nucleoplasmic and perinuclear localization in ESC nuclei, and upon differentiation its nuclear periphery localization decreases [34•]. Polycomb target genes, which are marked with H3K27me3, are mainly repressed in ESCs and start to be appropriately transcribed in later developmental stages. Therefore, in this model we hypothesize that polycomb target genes preferentially localize at the nuclear periphery (perhaps by interacting with lamin B) and are kept silenced.

acetylation [11•,43], and inhibition of histone deacetylation in ESCs prevents differentiation [43,44]. More specifically, H3K9ac was shown to be more prevalent in ESCs than differentiated cells [13••,45•,46•,47], in line with a globally decondensed chromatin. Other histone modifications associated with active chromatin, including H3K14ac, H3K4me3, H3K36me2 and H3K36me3, are also enriched in ESCs compared to their derived NPCs [13••]. Consistently, several repressive and heterochromatic marks were found to increase during ESC differentiation. These include H3K9me1, H3K9me2, H3K9me3 [11•,13••,43,47,48••] and HP1α [35,47], but not H4K20me2 [13••]. Importantly, epigenetic markings seem to correlate with pluripotency. In highly pluripotent hESCs, H3K9me3 is significantly diffuse while it is highly enriched in heterochromatin foci in less pluripotent hESCs and in differentiated cells [45•]. These data demonstrate that ESCs possess a distinct, functionally relevant, epigenetic landscape, that may correlate with pluripotency.

The open chromatin structure and the epigenetic properties of ESCs are likely due, at least in part, to the activity of chromatin-modifying enzymes [49]. One group of chromatin modifiers — ATP-dependent chromatin-remodeling proteins — is highly abundant in undifferentiated ESCs [13••,50]. Recent reports highlight several chromatin-remodeling proteins in ESC biology. These include BAF250B-associated complex [51]; Bptf [52]; BAF155 [53–55] and Chd1, a euchromatin remodeler associated with H3K4 tri-methylation that controls open chromatin and pluripotency in ESCs [56••].

One of the direct consequences of open chromatin in ESCs seems to be global pervasive transcription [13••]. Whole-genome tiling array technology revealed elevated transcription of intronic and non-genic regions in undifferentiated ESCs. The global levels of nascent RNA and mRNA are almost two-fold higher in mESCs than their differentiated counterparts, and tissue-specific genes are transcribed at very low levels in the undifferentiated state [13••]. This global hyper-transcription may be functionally significant for maintaining pluripotency and for proper differentiation, or may merely be a byproduct of an open chromatin conformation. However, the fact that many of the transcripts reside in intronic or intergenic regions may imply that at least some may act as non-coding RNAs that regulate pluripotency [57–60].

Genome organization in embryonic stem cells

The spatial organization of the genome in the nucleus is believed to provide an additional level of transcriptional regulation [61–64]. Mammalian genomes are highly organized in the three-dimensional space of the nucleus. In interphase, chromosomes occupy nonrandom locations, termed chromosome territories (CTs), and it was shown in different models that single genes or gene clusters can be distributed non-randomly within the nucleus or relative to their CT [65–67]. In all likelihood, these preferential positions play a regulatory role since some of these positions can change among different cell types, different tissues and during development [68–70]. Therefore, the characterization of genome organization in ESCs and in pluripotent cells undergoing the first steps of commitment or differentiation is highly intriguing and has recently begun.

Chromosome positioning

The genome of ESCs, similar to differentiated cells, is already organized in CTs. Moreover, the chromosome-positioning pattern observed in different hESC lines resembles that of differentiated cells. Gene-rich chromosomes, such as chromosome 19, seem to have a more central nuclear location than gene-poor chromosomes, such as 18, which is more peripheral [71]. The radial arrangement of CTs 6, 8, 10, 12, 15, 17 and 19 is also similar between pluripotent and differentiated hESCs [14,45•]. Nevertheless, the chromosome arm 12p, which harbors the pluripotency-related gene Nanog, seems to be slightly closer to the nuclear center in hESC compared to lymphoblastoid cells [71]. Although the distribution of somatic chromosomes do not appear to change significantly, one evident change in the genome organization of ESCs is the central position of the two X chromosomes in female cells, one of which becomes inactivated during differentiation and relocates to the nuclear periphery [45•,72]. Although these studies do not show any dramatic regulation of CTs in ESCs, they provide strong evidence that CTs are already established, with preferred locations, in ESCs.

Telomeres and centromeres

In hESCs, not unlike somatic cells, telomeres are distributed mostly in the center of the nucleus [71], again showing no distinct pattern. In contrast, the global proportion of

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centromeres not associated with either the nuclear periphery or the nucleolus is significantly higher in hESCs when compared with differentiated cell types [71] (Figure 1d). More specifically, centromeres 1, 5, and 19 relocate centrally and become clustered around nucleoli following hESC differentiation, whereas centromeres 10, 15 and 17 are redistributed at, or near the, nuclear envelope [45•]. These data demonstrate that rearrangement of pericentric heterochromatin domains takes place during hESC differentiation, likely reflecting the rearrangement of heterochromatin foci, discussed above.

Gene loci

The spatial nuclear organization of single genes in ESCs was also explored. As shown for the chromosome arm 12p, Nanog locus was found to be slightly more internal in nuclei of hESCs than in lymphoblastoid cells [71]. However, the chromosomal locus of the pluripotency-related gene Oct4 and the locus of the proto-oncogene c-Myc loci, which was identified as one of the original reprogramming factors, did not show any radial nuclear repositioning after differentiation [14,73]. In mouse as well, three of the eight tested loci did not reveal significant differences in their radial nuclear positioning (including Oct4) between ESCs and induced differentiated macrophages, but β-globin, lysosyme and myb gene loci showed significant relocations [73]. These studies suggest that ESC differentiation entails spatial regulation of specific gene loci, although so far no general rule seems to be emerging from the different studies.

Gene loci are often analyzed in the context of their CT rather than their nuclear spatial distribution, potentially adding another dimension to genome organization. In humans, the Nanog and c-Myc loci are distributed well within and at the edge of their corresponding CTs, respectively, and these positions remain unaltered following differentiation. In contrast, the Oct4 locus is positioned externally to its CT in hESCs, whereas in lymphoblastoid cells and in differentiated cells it is mainly found at the CT boundary [14,71]. However, the Oct4 locus (in relation to its CT) is more internal in mESCs than in hESCs [73], resembling the situation in differentiated cells. One possible explanation for the discordance between Oct4 positioning relative to its CT in mouse and human ESCs could be the differential expression of the neighboring genes, which differ between the two species [73].

In hESCs, this particular locus was shown to loop out from its CT, possibly reflecting increased accessibility of the locus to transcriptional factories [14]. In mouse ESCs, looping out of the Hox cluster from within its CT, for example, was also shown to correlate with transcriptional activity of the residing genes [74]. However, the correlation between looping out and expression has more recently been shown to be more complex. For example, when looping out from CTs was induced by the activation of Hox loci during differentiation, there was no effect on the expression level of the different genes located at or near the looping locus, suggesting that looping out of a gene from its CT is not sufficient to activate its transcription [75].

Interestingly, Oct4 in mouse regulates the higher chromatin structure of the Nanog locus, which contains several co-regulated pluripotency-related transcription factors [76•]. Oct4 repression leads to elevated levels of H3K9me3 and decreased binding of RNAPII and of the insulator protein CTCF at the Nanog locus, resulting in the downregulation of Nanog mRNA [76•]. More importantly, chromosome conformation capture (3C) analyses before and after Oct4 depletion demonstrated the disappearance of several contacts within the Nanog locus, which play important regulatory roles [76•]. These data show that Oct4 controls higher order chromatin structure of Nanog locus, suggesting potential cross-talks between pluripotency-related genomic locations.

Conclusions and future perspectives

Chromatin regulation and genome organization are steadily moving into central stage in search for mechanisms that control pluripotency. The recent burst of knowledge describing the genome of ESCs, from single gene positioning to the global epigenetic landscape, draws an increasingly clear picture linking chromatin with pluripotency. In addition to the growing list of chromatin-related genes and proteins that control stem cell maintenance and/or differentiation, several emerging observations call for studying the composition of chromatin itself, namely, histones and histone variants. The histone variant H2A.z, for example, controls ESC differentiation [77], and several others seem to be differentially regulated in ESCs [78]. Core histone proteolysis was also reported in ESCs. The H3 N-terminus tail is cleaved during ESC differentiation [79••], leaving an exposed core histone which is refractory to post-translational modifications such as methylation on lysine 4 and/or acetylation or methylation on lysine 9. Modern technologies ranging from high-throughput sequencing, automated multi-dimensional proteomic approaches and 5C analyses (ChIP-chip 3C), to name a few, will no doubt reveal more of the mysteries of pluripotent cells.

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