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Epigenetic regulation of open chromatin in pluripotent stem cells

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Abstract

The recent progress in pluripotent stem cell research has opened new avenues of disease modeling, drug screening, and transplantation of patient-specific tissues that had been unimaginable until a decade ago. The central mechanism underlying pluripotency is epigenetic gene regulation; the majority of cell signaling pathways, both extracellular and cytoplasmic, eventually alter the epigenetic status of their target genes during the process of activating or suppressing the genes to acquire or maintain pluripotency. It has long been thought that the chromatin of pluripotent stem cells is globally open to enable the timely activation of essentially all genes in the genome during differentiation into multiple lineages. The current article reviews descriptive observations and the epigenetic machinery relevant to what is supposed to be globally open chromatin in pluripotent stem cells. This includes microscopic appearance, permissive gene transcription, chromatin remodeling complexes, histone modifications, DNA methylation, noncoding RNAs, dynamic movement of chromatin proteins, nucleosome accessibility and positioning, and long-range chromosomal interactions. Detailed analyses of each element, however, have revealed that the globally open chromatin hypothesis is not necessarily supported by some of the critical experimental evidence, such as genome-wide nucleosome accessibility and nucleosome positioning. Further understanding of the epigenetic gene regulation is expected to determine the true nature of the so-called globally open chromatin in pluripotent stem.

Introduction

It is generally accepted that chromatin in pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced PSCs (iPSCs), is globally decondensed or “open” so that the cells can readily express many genes as they become necessary for differentiation into many lineages. Although the idea of open chromatin in PSCs is not novel, the recent progress in PSC biology, in particular the creation of iPSCs,¹ uncovered new pieces of evidence that

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potentially explain mechanistic links between pluripotency and open chromatin. A major impetus for this progress has been various types of genome-wide surveys, represented by the international effort of the ENCODE (Encyclopedia of DNA Elements) project.^{2–6} As of December 2013, this project had uncovered a wide spectrum of epigenetic modifications, including histone modifications, DNA methylation, transcription factor binding, chromatin accessibility, and long-range chromosomal interactions, in 338 human cell types and 86 mouse cell types, including ESCs. In addition, the U.S. National Institutes of Health (NIH) Roadmap Epigenomics Consortium has published genome-wide analyses of histone modifications, DNA methylation, and chromatin accessibility with an emphasis on stem cells, including ESCs and iPSCs, and their differentiated derivatives.^{7,8} Supported by the invention of sophisticated tools for chromatin analyses and bioinformatics, the explosive advance of this field is expected to continue at an accelerated pace. Because epigenetic regulation in PSCs has been discussed in many review articles,^{9–17} the current article will focus on epigenetic regulation that is directly relevant to globally open chromatin in PSCs. Methodological aspects of genome-wide epigenetic studies have also been reviewed elsewhere¹⁸ and will not be covered here.

Chromatin decondensation at the microscopic level

The initial evidence for the presence of open chromatin in undifferentiated cells came from morphological studies at the microscopic level. Long before mouse ESCs were created in 1981¹⁹ and pluripotency became a major cell biological research field, gradual, unidirectional, and global chromatin condensation was well documented with Wright stain during differentiation of hematopoietic cells.^{20,21} Densely stained areas (heterochromatin) within the nucleus gradually increase and become coarse and granular during differentiation of cells of each hematopoietic lineage. This observation is even more conspicuous with transmission electron microscopy, which shows an increase of electron-dense material that is initially scattered in local patches and eventually occupies wide areas, sometimes more than half of the nuclear space.^{20–22} Electron microscopy also displays fine, evenly distributed granules in the ESC nucleus that become more irregularly clustered after differentiation.²³ Although the electron-dense materials represent poorly characterized aggregations of DNA, protein, and RNA, and their precise functions remain unknown, they are generally interpreted to represent condensed chromatin and provide evidence that condensed chromatin increases during differentiation of undifferentiated cells, including PSCs.

In addition to the nucleus-wide chromatin condensation, centromeres have served as a model structure that undergoes condensation during differentiation of PSCs. Round centromeric heterochromatin looks more diffuse in ESCs than in neural progenitor cells differentiated from the same ESCs when stained with an antibody against the heterochromatin protein HP1a, which is highly enriched in centromeres.²⁴ The total level of HP1a also increases during differentiation. Fluorescence *in situ* hybridization (FISH) of the major satellite DNA sequence, one of the dominant DNA sequences of centromeres, also demonstrates more diffuse distribution in ESCs than in neural progenitor cells.²⁴

Permissive transcription

Another observation frequently cited as evidence for open chromatin in PSCs came from transcriptome studies comparing ESCs and differentiated cells.^{23, 25} ESCs contain twice as much total RNA and mRNA, normalized to the amount of DNA, as neural progenitor cells.²³ Microarray studies indicated that whereas differentiated cells generally express 10–20% of all mRNA species, ESCs express 30–60% of all mRNA species.²⁵ The mRNAs expressed in ESCs include those of many tissue-specific genes that do not appear to be necessary for maintaining the undifferentiated state of ESCs. Although some of the tissue-specific genes are expressed at very low levels,²³ others are translated into proteins,²⁶ indicating that they are not artifacts of detection by sensitive PCR. This permissive transcriptional environment is not limited to PSCs; hematopoietic stem cells also express non-hematopoietic genes, which are gradually downregulated during differentiation.^{27,28}

Although these findings may fit the interpretation of uncontrolled leaky transcription due to open chromatin, definitive evidence is still missing. It is also possible that the primary cause of the prevalent transcription is as-yet-uncharacterized functions of the transcription machinery rather than the chromatin structure as a substrate for transcription. Chromatin immunoprecipitation and DNA microarray (ChIP-chip) and genome-wide nuclear run-on experiments indicated that RNA polymerase II is paused at the promoters of 40–50% of all the protein-coding genes without elongation of mRNA in ESCs, suggesting that post-initiation processes are the rate-limiting steps in transcription.^{25,26} Although this high frequency of paused polymerase may look to be a promising explanation for the permissive expression in ESCs, similar results have in fact been obtained with differentiated cells, such as hepatocytes, B cells, and embryonic fibroblasts.^{29,30} Thus, the link between permissive transcription and polymerase II pausing has not been established.^{29, 30}

Chromatin remodeling complexes

Chromatin remodeling complexes potentially play key roles in the permissive transcription in ESCs. The complexes use ATP to relax DNA-histone interactions and thereby increase the fluidity of chromatin structure.^{31,32} They comprise 4 families (SWI/SNF [switching defective/sucrose nonfermenting], also called BAF [Brg/Brahma-associated factor]; ISWI [imitation switch]; CHD [chromodomain, helicase, and DNA binding]; and INO80 [inositol requiring 80]) and are involved in almost all types of chromatin activities, including transcription, DNA repair, and DNA replication.^{31,32} Not surprisingly, many subunits of the complexes are essential for the maintenance of pluripotency and proliferation of ESCs, as recently reviewed.¹⁰ In addition, many of the subunits are highly expressed in ESCs. Efroni et al. reported that out of 25 detectable subunits, 20 are significantly downregulated during differentiation of ESCs to neural progenitor cells and 5 are slightly upregulated.²³

Chd1, the catalytic subunit of the CHD complex, is necessary for the maintenance of open chromatin in ESCs.³³ Genome-wide binding sites of Chd1 correlate well with those of trimethylation of lysine 4 of histone H3 (H3K4me3; a marker for active genes) and RNA polymerase II, suggesting a role for Chd1 in gene activation. Knockdown of Chd1 in ESCs converts diffuse centromeric heterochromatin into more compact structures, as observed

with the immunofluorescence staining of H3K9me3 (a marker for suppressed genes), which is enriched at centromeric heterochromatin, and its binding protein HP1 γ . However, Chd1 knockdown decreases the expression of only 26 genes in addition to *Chd1* itself, potentially due to functional redundancy with other chromatin remodeling complexes. Although ESCs with Chd1 knockdown can maintain an undifferentiated state, the cells lose the ability to differentiate into primitive endoderm, and they tend instead to differentiate into neural lineages, indicating that Chd1 is necessary for the maintenance of pluripotency.

esBAF is an ESC-specific SWI/SNF complex that is also required for the maintenance of pluripotency in ESCs.^{34,35} esBAF colocalizes with the 3 master transcription factors of pluripotency—Oct4, Sox2, and Nanog—in the ESC genome and is essential for sustaining the core pluripotency transcriptional network.³⁴ Although Brg1, the catalytic (ATPase) subunit of esBAF, is widely expressed in differentiated cells as well, esBAF is characterized by the presence or absence of specific subunits. For example, the BAF170 subunit is absent from esBAF, whereas it is present in other SWI/SNF complexes; ESCs overexpressing BAF170 proliferate less competitively than non-transduced cells when these 2 cell types are co-cultured. The BAF155 subunit is present in esBAF and its depletion leads to decreased cell proliferation, downregulated *Oct4* expression, and increased apoptosis.³⁶ This subunit is required for heterochromatin formation during differentiation of ESCs but its role in open chromatin remains unknown.³⁶

Histone modifications

Histones of ESCs are characterized by an increased amount of modifications that are involved in gene activation (H3K4me3, acetylation of lysine 9 on histone H3 [H3K9ac], H3K14ac, and H4ac) and a decreased amount of the modification that is a hallmark for gene suppression (H3K9me3) compared with those in differentiated cells.^{23,24,37} In addition, large chromosomal regions of up to 4.9 Mb enriched with H3K9me2 called LOCKs (large organized chromatin K9 modifications) are markedly decreased in ESCs compared with differentiated cells³⁸: LOCKs cover 4% of the genome in ESCs, in contrast to 31% in differentiating ESCs and 46% in liver cells. Importantly, genes located within LOCKs are generally suppressed, providing another example of ESC-specific open chromatin. These specific increases and decreases of histone modifications are potentially relevant to the permissive transcription in ESCs.

Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2) are two of the most extensively studied histone modifying complexes that are involved in gene suppression in general.^{39–42} PRC2 contains Suz12, Ezh2, Eed, and RbAp46/48 as core components and induces H3K27me2 and H3K27me3 through the methyltransferase activity of Ezh2. H3K27me2 and H3K27me3 recruit PRC1, which induces monoubiquitylation of H2AK119 with the ubiquitin ligases Ring1A and Ring1B in PRC1; however, the link between PRC2 and PRC1 remains controversial.⁴² H3K27me3 is a well-established marker for suppressed genes, typically existing at the promoters and transcriptional initiation sites of these genes. Although several mechanisms have been proposed, the exact mechanism of suppression remains elusive.

PRC2 binds to 200–500 genes in the ESC genome, many of which encode transcription factors important for differentiation.^{43,44} These suppressed genes are marked with H3K27me3 in ESCs and become activated during differentiation of ESCs concomitant with the loss of PRC2. Knockout of the PRC2 components indicated that PRC2 is not essential for ESCs to maintain an undifferentiated state despite global depletion of H3K27me3; however, PRC2 is required for proper differentiation of the cells.^{45–47}

Many promoters in ESCs are marked by the co-existence of H3K4me3 (active genes) and H3K27me3 (suppressed genes), which is called a bivalent mark. H3K27me3 is lost when the gene is activated, and H4K4me3 is lost when the gene remains suppressed during differentiation. Based on these findings, these marks were initially thought to indicate pluripotency-specific genes in ESCs that are poised to be activated when they become necessary during differentiation.^{48,49} However, subsequent studies showed that a bivalent mark is not unique to ESCs,^{50–52} and H3K27me3 is not necessary to maintain the undifferentiated state of ESCs.⁴⁵ The functions and molecular mechanisms underlying bivalent marks were thoroughly discussed in a recent review article.⁴⁹

DNA methylation

The promoters of suppressed genes are frequently marked by methylation of cytosine (5-methylcytosine or 5mC) at the CpG sequence.^{53,54} Although, as mentioned earlier, ESCs express more mRNA species than differentiated cells, the global level of 5mC at CpG sites is similar in ESCs and differentiated cells.^{55,56} However, 5mC at non-CpG sites is uniquely abundant in ESCs^{56, 57}; whereas both human fibroblasts and ESCs contain 45 million 5mCs at CpG sites, ESCs harbor 17 million 5mCs at non-CpG sites and fibroblasts harbor almost none.⁵⁶ The non-CpG 5mC is enriched in gene bodies and disappears upon differentiation of ESCs; however, the functions of non-CpG 5mC, including their connection to open chromatin, are largely unknown.

New 5mCs are established by the *de novo* DNA methyltransferases Dnmt3a and Dnmt3b. Once established, 5mC is maintained during DNA replication by Dnmt1. ESCs prepared from *Dnmt1* knockout mice and single and double *Dnmt3a* and *Dnmt3b* knockout mice can remain undifferentiated despite a significant loss of 5mC, although proper differentiation of ESCs with *Dnmt1* knockout and *Dnmt3a/b* double knockout is disrupted.^{58–60}

All three Ten-eleven Translocation proteins (Tet1, Tet2, and Tet3) sequentially oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC).^{61–63} Thymine DNA glycosylation and base excision repair can convert 5fC and 5caC to cytosine, resulting in demethylation of the original 5mC.^{61–63} Whereas Tet1 and Tet2 are highly expressed in ESCs and downregulated during differentiation, Tet3 is upregulated during differentiation.^{64,65} The level of 5hmC, which is primarily maintained by Tet1 and Tet2 in ESCs, also decreases during differentiation of the cells.^{64–66} 5hmC is enriched within exons, enhancers, and promoters of active and inactive genes; thus, unlike 5mC, 5hmC is not tightly correlated with the status of gene activity.⁶⁷ Single knockout of *Tet1* or *Tet2*, and double-knockout of *Tet1* and *Tet2* do not compromise the undifferentiated state of ESCs, although differentiation of the *Tet1* knockout and the double knockout ESCs

is skewed toward endoderm and trophoctoderm.^{64,66,68} The double-knockout completely depletes 5hmC; however, gene expression is altered 1.5-fold or more only in 500 genes.⁶⁶ Overall, 5mC and 5hmC, as well as their responsible enzymes, are not needed for ESCs to proliferate and remain undifferentiated. However, ESCs cannot differentiate into the full spectrum of lineages without these enzymes, which indicates the essential roles for these DNA modifications for maintaining the pluripotency of ESCs. Whether or not ESCs can retain open chromatin without these modifications requires further study. However, the limited differentiation potential suggests that open chromatin is lost locally at relevant genes in the absence of these DNA modifications.

Noncoding RNAs

microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) are also involved in the regulation of chromatin structure in PSCs. miRNAs are generally 20–21 bases in length and bind to target mRNAs, most commonly at the 3' untranslated region (UTR), which leads to degradation or translational inhibition of the target mRNAs.⁶⁹ miRNAs indirectly control chromatin structure through modulating the protein level of various chromatin-modifying proteins. For example, the miR-290 cluster, which contains miR-290 through miR-295, maintains the levels of Dnmt3a and Dnmt3b potentially by repressing inhibitors of Dnmt3, such as Rbl2, in ESCs.⁷⁰ In contrast, miR-29b represses Dnmt3a and Dnmt3b and contributes to the formation of iPSCs.⁷¹ The miR-125 and miR-181 families provide another example of the miRNAs-chromatin connection in PSCs. These miRNAs are induced during differentiation of ESCs and downregulate their target Cbx7, a pro-self-renewal component of PRC1. Loss of Cbx7 de-represses pro-differentiation components of PRC1, such as Cbx2 and Cbx4.^{72,73}

lncRNAs are defined as RNAs longer than 200 bases that are not mRNA, rRNA, or tRNA.^{74,75} PSCs express unique lncRNAs that bind to specific chromatin modifying enzymes and recruit these proteins to the binding sites of the lncRNAs in the genome. Guttman et al. found that 74 ESC-enriched lncRNAs are bound to many chromatin modifying proteins, including PRC2, Jarid1c H3K4 demethylase, and Eset H3K9 methyltransferase.⁷⁶ Two other ESC-enriched lncRNAs called lncRNA_ES1 and lncRNA_ES2 also associate with the PRC2 component Suz12, promoting the maintenance of pluripotency of ESCs.⁷⁷ These examples demonstrate indirect roles of lncRNAs in the regulation of chromatin structure primarily through the targeting of chromatin modifying proteins to specific genomic loci. Because the function of lncRNAs is so diverse, future study may discover lncRNAs that directly alter chromatin structure through the interaction with DNA or histone.

Dynamic movement of chromatin proteins

Chromatin proteins within the nucleoplasm move more rapidly in ESCs than in differentiated cells, as was shown with the fluorescence recovery after photobleaching (FRAP) technique.²⁴ In a typical FRAP experiment, a cDNA encoding a chromatin protein is fused with a fluorescent protein gene, most commonly green fluorescent protein (GFP), and transfected into tissue culture cells. A small area within a nucleus that expresses the

GFP-fusion gene is exposed to high-intensity laser light to irreversibly bleach the GFP signal in the area. The time course of the loss and recovery of the GFP signal in the area is then followed. The recovery of the GFP signal indicates the migration of GFP-fused protein from the surrounding areas, which reflects the size of the freely movable pool of the protein, the release of the proteins from their binding sites in the surrounding areas, and the speed of the migration, among other factors.⁷⁸ When GFP was fused to HP1 α , the linker histone variant H1 $^{\circ}$, and core histones, Meshorer et al. found that the GFP signal for all these proteins recovers more rapidly in ESCs than in neural progenitor cells.²⁴ Salt extraction of these proteins from chromatin also indicates weaker binding of the proteins to chromatin in ESCs than in neural progenitor cells. These results are interpreted to indicate that ESCs harbor more dynamic chromatin than differentiated cells. The recovery of the GFP signal of GFP-histone H1 is facilitated in ESCs by 1 of 3 manipulations: increased histone acetylation, decreased H3K9 methylation, and decreased lamin A, providing mechanistic connections between protein movement and epigenetics.⁷⁹ The mobility of histone H1 is not affected by reductions in DNA methylation induced by chemicals or by reductions in nucleosome repeat length, which is the average distance between two adjacent nucleosomes, by depletion of linker histone H1 variants.

In addition to the movement of individual chromatin proteins, chromatin vibrates as a mass at a frequency of 10 to 100 Hz in ESCs (called breathing).⁸⁰ This was detected as a back-and-forth shift of the areas densely stained with the DNA dye Hoechst 33342 within a distance of 90 to 100 nm. The chromatin breathing becomes weak as early as 3 days after initiation of ESC differentiation and is completely lost by 15 days after initiation of differentiation. Furthermore, the breathing is dependent on ATP, which might be used as a substrate for chromatin remodeling complexes, although this possibility has not been experimentally tested.

Nucleosome accessibility and positioning

Increased accessibility of chromatin to DNase I or restriction enzymes is commonly used as a measure of open chromatin. The pattern of genome-wide distribution of DNase I hypersensitivity sites (DHSs) has been compared between ESCs and many differentiated cell types.^{81,82} DHSs are generally depleted of nucleosomes and are typically observed at gene regulatory regions, including promoters, enhancers, and insulators. The total number of DHSs is similar in ESCs and 6 differentiated cell lines.⁸² This result is consistent with a finding independently obtained with chromatin fragmentation by sonication in a FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) experiment.⁸² When an open chromatin site is defined as a site that is hypersensitive to both DNase I and sonication, cell type-specific open chromatin sites are usually located at the regulatory regions of key transcription factor genes that define the identity of the cell type. For instance, there are many open chromatin sites around *Oct4* and *Nanog* that are specific to ESCs.⁸² However, the number of open chromatin sites defined by these techniques is not necessarily higher in ESCs than in differentiated cells, which contradicts the notion of globally open chromatin in ESCs.

In a related approach, nucleosome positioning has been compared between different cell types. Positioning of nucleosomes in the genome is determined by many factors, including DNA sequence and the presence of chromatin remodeling complexes and transcription factors.⁸³ A genome-wide study of nucleosome positioning detected a difference in nucleosome repeat length: 186 base pairs in ESCs and 191–193 base pairs in differentiated cells.⁸⁴ Whether this seemingly subtle difference has a functional consequence in gene expression awaits further investigation.

Long-range chromosomal interactions

Many types of cells display specific patterns of intra- and interchromosomal interactions as another layer of epigenetic gene regulation. These chromosomal interactions are studied with the techniques called chromosome conformation capture (3C) and its genome-wide variants.⁸⁵ Although these techniques differ in the comprehensiveness of the coverage of interactions, in principle they involve crosslinking of interacting chromatin, fragmentation of DNA with restriction enzymes, ligation of nearby DNA fragments, and identification of ligated DNA sequences with PCR or next-generation sequencing technology. Results obtained with these techniques can be independently verified with FISH, which can detect interacting DNA sequences as the colocalization of two fluorescent signals.

Kagey et al. were the first to demonstrate a physical interaction between an enhancer and a promoter via the formation of a chromosomal loop within the *Oct4* and *Nanog* pluripotency gene loci in mouse ESCs.⁸⁶ The interaction is mediated by the binding of cohesin, which promotes sister chromatid cohesion, and the coactivator complex called mediator to the enhancers and promoters. Later, a cohesin-dependent enhancer–promoter interaction was also reported at the *OCT4* locus in human ESCs.⁸⁷ Extending these studies of local chromosomal interactions, a genome-wide study unraveled numerous chromosomal interactions in PSCs, some of which are specific to pluripotency as shown by the loss of the interactions after differentiation of the cells.^{88–93} These interactions are generally dependent on the presence of mediator, cohesin, and the CCCTC-binding factor (CTCF), which serves as an insulator dividing 2 chromosomal domains.⁹⁴ In addition, the pluripotency transcription factors Oct4, Sox2, and Klf4 mediate the chromosomal network between pluripotency genes and non-pluripotency genes. These findings suggest that PSCs harbor a sophisticated network of chromosomal interactions to organize co-regulated gene expression; however, it remains to be studied how many of the observed chromosomal interactions have functional roles.

Chromosomes are also associated with lamin B1, a major meshwork component of the nuclear lamina underlying the nuclear envelope, at more than 1000 loci in ESCs.⁹⁵ The genes located within the chromosome domains attached to the nuclear envelope via lamin B1 tend to be transcriptionally inactive; the genes encoded in chromosomal loops that protrude into the nuclear interior from the nuclear envelope tend to be transcriptionally active. This conformation dramatically changes during differentiation of ESCs depending on the activity of each gene. Overall, these studies underscore the potential significance of large-scale chromosome–chromosome and chromosome–lamin B1 interactions in the regulation of pluripotency. A critical question relevant to the current article is how to

reconcile the open and dynamic chromatin structure in ESCs with the intricate chromosome meshwork that potentially limits chromatin dynamism. Chromosomal interaction is one of the most rapidly progressing areas in the research of stem cell epigenetics, and we expect that this question will be addressed at some point.

Future perspectives

In summary, the notion of globally open chromatin in PSCs is supported by some, but not all, evidence, and we await definitive data. The supportive evidence includes morphological observations, permissive transcription, dynamic movement of chromatin proteins, and chromatin breathing as phenomenological findings (Table 1). Specific epigenetic markers, such as the total amount of various histone markers and the presence of specific chromatin remodeling complexes, also support the idea of open chromatin. Although 5mC, 5hmC, and H3K27me3 are not necessary for ESCs to remain undifferentiated, they potentially contribute to the maintenance of open chromatin in undifferentiated ESCs because ESCs display skewed differentiation patterns without these modifications; however, this interpretation requires verification. In contrast, indicators of physically relaxed chromatin, such as nucleosome accessibility, nucleosome repeat length, and chromosomal interactions, have not provided decisive evidence supporting the open chromatin interpretation. In fact, the studies of nucleosome accessibility, which is one of the parameters most relevant to open chromatin, using 2 independent approaches indicated that there is no significant difference between ESCs and differentiated cells. The lack of physical evidence for the globally open chromatin suggests that the difference of chromatin structure between PSCs and differentiated cells is more subtle than anticipated. Overall, further study is required to uncover the exact structure of what is supposed to be globally open chromatin in PSCs that can be explained by specific molecules unique to ESCs.

Another important issue in the context of epigenetics and pluripotency is the true nature of pluripotency itself. Oct4, Nanog, and Sox2 have long been thought to maintain pluripotency through suppression of differentiation-specific genes because they bind to regulatory regions of many lineage-specific transcription factor genes.^{96,97} However, a cluster of recent studies indicated that pluripotency is in fact acquired and maintained through the tug of war of differentiation toward opposing fates (Oct4 to mesendoderm, Nanog to endoderm, and Sox2 to ectoderm) and the resulting mutual inhibition of differentiation into any specific germ layer cell types.^{98,99} For example, Oct4 promotes mesendoderm differentiation and suppresses ectodermal differentiation of ESCs, whereas Sox2 induces ectodermal differentiation and inhibits mesendodermal differentiation.^{100,101} Indeed, iPSCs can be created by transduction of lineage-specific transcription factor genes instead of pluripotency factor genes.^{102, 103} Therefore, pluripotent chromatin does not necessarily indicate chromatin purposefully kept half-open for future differentiation. Rather, it could be more appropriate to envision a composite of different sets of half-activated genes, each driven by a master differentiation transcription factor.

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Abbreviations

DHS	DNase I hypersensitivity site
ESC	embryonic stem cell
FISH	fluorescence <i>in situ</i> hybridization
GFP	green fluorescent protein
iPSC	induced pluripotent stem cell
5mC	5-methylcytosine
PRC1 and 2	Polycomb Repressive Complex 1 and 2
PSC	pluripotent stem cell
Tet	Ten-eleven translocation

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Summary of epigenetic markers relevant to globally open chromatin in pluripotent stem cells.

Table 1

Epigenetic marker	Pluripotent stem cell (PSC)-specific feature	Relevance to globally open chromatin
Morphological appearance	Diffuse centromeric heterochromatin	Suggestive
Permissive gene transcription	Fine and homogeneous electron-dense materials	Suggestive but identity of the electron-dense material remains unknown
Chromatin remodeling complexes	More mRNA species	Suggestive but other interpretations possible
	20 complexes more abundant	Can relax DNA-histone interactions
	Chd1	Relaxes centromeric heterochromatin
	esBAF	Necessary for dynamic movement of HP1 γ
Histone modifications	H3K4me3, H3K9ac, H3K14ac, and H4ac: PSCs > differentiated cells	Unknown
	LOCKs (H3K9me2): PSCs < differentiated cells	Suggestive
	H3K27me3	Suggestive
DNA methylation	Number of 5mC at CpG: PSCs = differentiated cells	Required for pluripotency but link to open chromatin is unknown
	Number of 5mC at non-CpG: PSCs > differentiated cells	Required for pluripotency but link to open chromatin is unknown
	Number of 5hmC: PSCs > differentiated cells	Unknown
miRNAs and lncRNAs	Some are specifically expressed in ESCs	Required for pluripotency but link to open chromatin is unknown
Dynamic movement of chromatin proteins	FRAP: PSCs > differentiated cells	Involved in pluripotency but link to open chromatin is unknown
Chromatin breathing	Unique to PSCs	Sign of chromatin flexibility?
Nucleosome accessibility	PSCs = differentiated cells	Sign of chromatin flexibility?
Nucleosome repeat length	PSCs < differentiated cells	Contradictory to open chromatin
Long-range chromosomal interactions	Cell type-specific interactions exist	Unknown functional meaning
Chromosome-lamin B1 interaction	Cell type-specific interactions exist	Unknown
		Unknown