Chromatin plasticity in pluripotent cells

Shai Melcer and Eran Meshorer[1]

Department of Genetics, The Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Abstract

ESCs (embryonic stem cells), derived from the blastocyst stage embryo, are characterized by an indefinite ability for self-renewal as well as pluripotency, enabling them to differentiate into all cell types of the three germ layers. In the undifferentiated state, ESCs display a global promiscuous transcriptional programme which is restricted gradually upon differentiation. Supporting transcriptional promiscuity, chromatin in pluripotent cells is more 'plastic' or 'open', with decondensed heterochromatin architecture, enrichment of active histone modifications, and a hyperdynamic association of chromatin proteins with chromatin. During ESC differentiation, nuclear architecture and chromatin undergo substantial changes. Heterochromatin foci appear smaller, more numerous and more condensed in the differentiated state, the nuclear lamina becomes more defined and chromatin protein dynamics becomes restricted. In the present chapter we discuss chromatin plasticity and epigenetics and the mechanisms that regulate the various chromatin states, which are currently a central theme in the studies of stem cell maintenance and differentiation, and which will no doubt assist in delineating the secrets of pluripotency and self-renewal.

[1]*To whom correspondence should be addressed (email meshorer@cc.huji.ac.il).*

Introduction

In eukaryotes, DNA is arranged as a nucleoprotein superstructure termed chromatin. The basic unit of chromatin is the nucleosome, which comprises 147 bp of DNA wrapped around a core octamer of the highly conserved histone proteins H2A, H2B, H3 and H4 (two of each). A linear string of nucleosomes is organized via H1, a linker histone protein, into a tight helical organization, which is compacted further into complex high-order structures that are not yet fully understood. Chromatin structure is controlled by various protein complexes generally called chromatin modifiers, affecting DNA accessibility and DNA–protein interactions. Chromatin remodelling complexes are recruited to chromatin by a variety of signals, modifications on both DNA and histones, which together formulate an elaborate code, all in a specific context of DNA sequence, spatial organization, developmental stage and tissue specificity [1–4].

Chromatin plasticity therefore dictates many of the nuclear processes, including transcription, replication, cell-cycle kinetics, nuclear protein dynamics and chromatin modification, all of which ultimately facilitate or repress changes in transcriptional profiles, either locally or globally. Chromatin is the basic regulatory unit of life and, as such, it controls developmental and functional states of all cells, including pluripotency.

Pluripotent ESCs (embryonic stem cells) are derived from the ICM (inner cell mass) of the developing mammalian embryo at the blastocyst stage before implantation. When grown in culture, they are uniquely characterized by the ability to self renew indefinitely and by their potential to differentiate and give rise to all three germ layers, endoderm, mesoderm and ectoderm, and to ultimately become cells of all bodily tissues. Essentially, ESCs are an *in vitro* immortalization of what is actually a transient state *in vivo* (Figure 1).

Differentiation of ESCs into specialized cell types involves dramatic changes in gene expression patterns, tightly correlated with substantial alterations in DNA and histone-modification states and subsequent global changes in chromatin plasticity [5–7].

An interesting analogy was made between developmental potential and potential energy levels, in the context of epigenetic status and gene-expression profiles, comparing development with a downhill landscape [8,9]. According to Waddington's view, the pluripotent stage of ESCs (high developmental potential) reflects a topographically high-potential energy point, positioned up in the developmental landscape before a selection of lineage-committed 'valleys'. Thus the unipotent stage of differentiated cell types are viewed as low-potential energy points, positioned at the bottom of the valley, at the 'end of the road'. Multipotent cell types are therefore intermediate entities, having lost some potential energy when passing a major developmental junction, but left with enough potential energy to navigate between more inter-lineage developmental junctions ahead.

An exciting development in the field of ESCs is the ability to reprogramme somatic cells into iPSCs (induced pluripotent stem cells) with only four or

fewer ectopically expressed factors [10,11] (Figure 1). Induction of pluripotency in differentiated cells is perceived today as a key tool for regenerative medicine, allowing treatment of degenerative and other diseases without facing the problems of immunogenicity and ethical dilemmas of embryo manipulations. Thus it is extremely important to increase the efficiency of the iPSC generation process and extensively characterize the pluripotency and self-renewal of iPSCs in comparison with ESCs. As noted above, important in this regard are nuclear-related features, including gene-expression profiles, epigenetic states and subsequently chromatin structure and plasticity [12–15]. The common goal is to better understand what is required of a somatic cell in order to go up the developmental landscape and reposition itself before developmental junctions, first lineage-specific and then pluripotent ones, and how this can be safely and efficiently done *in vitro*.

In this chapter, we elaborate on transcriptional profiles and chromatin plasticity in ESCs, review their unique properties that distinguish them from
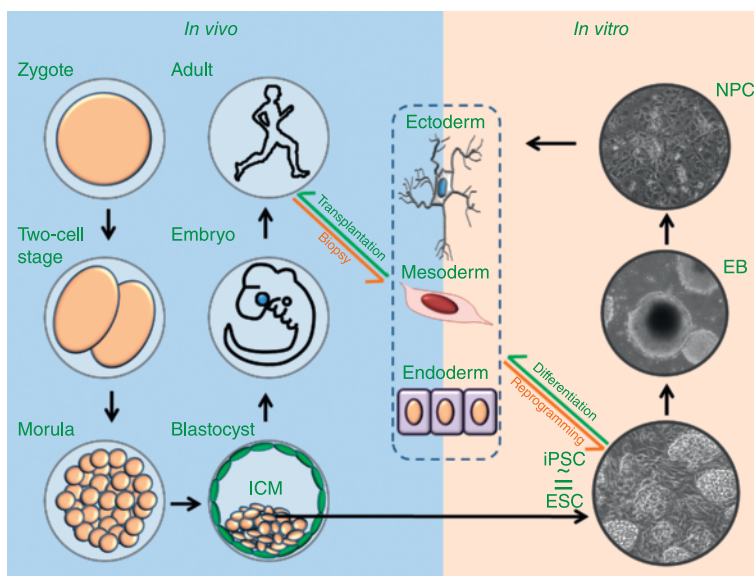

Figure 1. Pluripotency and differentiation
Embryonic development *in vivo* (left-hand panel, blue) begins with the fertilized zygote. After several divisions (two, four, eight cells etc.) leading to the morula, the first specification event occurs and the blastocyst is formed, comprising placenta precursors, the trophoectoderm cells (green) and the ICM (orange) which forms the embryo proper. When derived from the blastocyst and propagated *in vitro* (right-hand panel, orange), the ICM gives rise to ESCs. ESCs can be indefinitely grown in culture and conditioned to differentiate into cell types of all three germ layer lineages (central dashed box). Neuronal differentiation is carried out *in vitro* by suspended embryoid body (EB) formation, plating and further growth in defined media, resulting in NPCs and eventually differentiated neurons. When subjected *in vitro* to reprogramming factors, adult-derived differentiated somatic cells (central dashed box) become iPSCs (orange arrows). These patient-specific iPSCs can be manipulated according to clinical requirements, specifically differentiated *in vitro* and re-transplanted in the patient (green arrows).

those of differentiated cells and compare them with those of iPSCs. In both cases, we describe work that delineates the mechanisms underlying the differences and similarities between chromatin plasticity in pluripotent and differentiated cells.

Transcription profiles of pluripotent compared with differentiated cells

ESCs are distinct from differentiated cells both in gene expression patterns and transcriptional activity. Bearing in mind that pluripotency is no more (and no less) than the potential to become any cell type, gene expression in pluripotent cells can be viewed as a 'catalogue' and transcriptional activity as an open 'index' awaiting a choice of 'product/service' to be made in order for the appropriate page to open. When cells differentiate, some pages are closed and become less accessible to the 'readers'.

The master-regulator transcription factors Oct4, Sox2 and Nanog were found to associate with various groups of chromatin remodellers, histone modifiers and downstream transcription factors that activate pluripotent networks and at the same time act to repress tissue-specific pathways, which are crucial for the maintenance of pluripotency and self-renewal [16]. Intriguingly, this robust gene-expression regulation in ESCs is accompanied by highly promiscuous transcriptional activity characterized by extensive RNAPII (RNA polymerase II) occupancy and transcription initiations throughout the genome [17]. In differentiated cells, this transcriptional phenomenon is more restricted and has apparent tissue specificity. Overall, transcriptional activity in ESCs was found to be about twice as high as in differentiating cells such as NPCs (neuronal progenitor cells) [18]. Increased transcription seems to be pervasive rather than local, including permissive expression of tissue-specific genes and transcription of various non-coding regions, which are usually inactive in somatic cells [18].

Differentiation from ESCs to somatic cells goes through intermediate-stage cell types. These restricted stem/progenitor cells are multipotent and can differentiate further into a limited variety of specialized cells. Like pluripotent ESCs and fully differentiated somatic cells, these multipotent intermediates also have characteristic transcription profiles and subsequent gene-expression patterns which distinguish them from other cell types. It has been shown that, as cells differentiate, gaining function and losing potency, their gene-expression profiles become less and less promiscuous as whole gene clusters that are not required for the specific differentiation event shut down [18–23]. Thus pluripotent cells maintain full capacity to choose between lineages via a promiscuous transcription pattern, whereas low expression levels prevent them from actually performing any tissue-specific functions. This strategy has been called the 'open options' or 'just in case' hypothesis [20,24]. Differentiating cells focus their transcriptional pattern and gradually 'close' their options while committing to a specific lineage, then to a sublineage and finally to

a specialized somatic cell, fully functional, but with little or no options to change its fate.

Comparing gene expression of ESCs, iPSCs and partial iPSCs (cells that have undergone partial reprogramming and reached a state of multipotency) has repeatedly shown that pluripotent cells share similar, but not identical, patterns [11,25]. Both ESC and iPSC types express high levels of 'stemness' genes and very low levels of lineage-specific genes [11,25]. Also, pluripotent cells are inherently heterogeneous [26,27], but it seems that the reprogramming process is reaching an increasingly higher fidelity, resulting in more experimentally homogenous iPSC populations [28]. Whole transcriptome RNA-sequencing approaches could clarify similarities and differences between pluripotent cells derived *in vivo* and induced *in vitro*, such as previously compared in ESCs and differentiated cells using whole genomic tiling arrays [18], and elucidate further the transcriptional characteristics of pluripotency. If pan-genomic global transcription promiscuity is indeed such a striking pluripotency marker, both in ICM-derived ESCs and *in vitro* engineered iPSCs, one might consider a suggested outlook on 'stemness' as a cell state rather than a cell type [24].

Chromatin plasticity in pluripotent cells

We have discussed transcriptional profiles characteristic of pluripotent, multipotent and somatic cells as the basis for differentiation potential. We now discuss chromatin plasticity as a basis for the distinct transcriptional profile in pluripotent cells and review its characteristics and the biophysical changes it induces in cells along the pluripotency–differentiation trail (Figure 2).
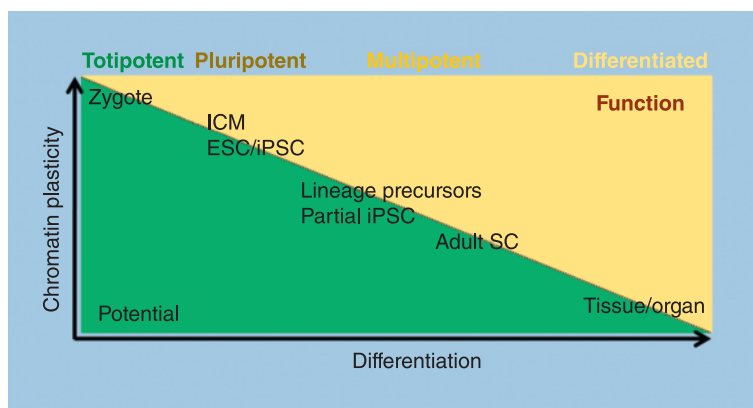

Figure 2. Chromatin plasticity and cellular potency–function relationship
Embryonic development is characterized by gradual loss of potency (green) and gain of function (orange). Decreasing throughout development, chromatin plasticity is in correlation with differentiation potential and in inverse correlation with specialized cellular function. Landmark cell types are positioned relatively according to their potency/function ratio and corresponding chromatin state.

ESCs feature 'open chromatin' that is defined architecturally and dynamically and is distinct from that in differentiating multipotent cells, such as NPCs [5] or MEFs (mouse embryonic fibroblasts) [29]. Overall, in ESCs, the portion of chromatin that is in the condensed state of heterochromatin is smaller compared with differentiating cells. Morphologically, ESC heterochromatin regions are large, diffused and amorphous compared with smaller, discrete and condensed foci in the differentiating NPCs [5,30] or primary MEFs [29]. Binding properties of chromatin-associated proteins, HP1 (heterochromatin protein 1), core histones and the linker histone H1, are also unique in pluripotent cells when compared with differentiating cells by FRAP (fluorescence recovery after photobleaching) and salt extraction assays. ESCs display chromatin hyperdynamics due to a small fraction of highly mobile HP1 and histones, whereas differentiation to NPCs results in partial immobilization of these dynamics indicators, observable as soon as 24 h after the onset of differentiation [5]. Furthermore, HP1 and H1 are loosely bound to chromatin in ESCs, as too are core histones, whereas in NPCs and primary MEFs, they display tighter binding, indicative of a substantially more condensed 'closed chromatin' formation [5,29]. This phenomenon is not without exception; histone H3.3 recovery dynamics were similarly slow in ESCs and NPCs [5], whereas histone H1.5 dynamics were similarly fast in ESCs and primary MEFs [29]. This suggests that chromatin protein dynamics are regulated and not just a biophysical outcome of the general chromatin condensation state. Experimenting with mutant ESCs displaying perturbed or enhanced histone binding established that proper chromatin plasticity – histone dynamics, turnover and availability – has functional implications and is essential for the differentiation process ([5], and S. Melcer, H. Hezroni, E. Rand, A. Skoultchi, M. Bustin and E. Meshorer, unpublished work).

It was further shown that the open chromatin formation and hyperdynamic plasticity correlate with the potency state in ESCs and multipotent cells [5] and this is coherent with gene-expression patterns and transcriptional profiles of pluripotent compared with differentiating cells [18]. Thus open chromatin facilitates the 'open options' promiscuous transcriptional strategy, exposing a great portion of the genome and marking it accessible to expression regulators and the transcriptional machinery. Once a differentiation choice has been made, chromatin undergoes a dramatic architectural 'shut down', losing potency and focusing on lineage-acquired functions. This differentiation-related chromatin-reorganization process evidently requires a free pool of chromatin proteins, such as histones, HP1 and chromatin remodellers.

Interestingly, the correlation between potency and chromatin plasticity is also found during early embryonic development in *Drosophila*. Histone H2B dynamics in the syncytial blastoderm is similar to that measured in mouse ESCs and it remains high until 1 h after cellularization [29]. H2B becomes significantly less dynamic with each nuclear division before cellularization and continues to tighten down until it reaches a stable highly immobile state, about 5 h after cellularization, parallel to an increase in chromatin rigidity [29]. Thus,

akin to mammalian differentiation, the fruitfly embryo exhibits gradual loss of chromatin plasticity upon acquisition of cell identity.

Taken together, these data show that pluripotent cells have a plastic open chromatin formation. This is true for several different studied systems at early embryonic development, including mammalian ESCs and *Drosophila* syncytial blastoderm. During embryonic development, when cells differentiate, lose potency and gain function, chromatin 'shuts down', it condenses and tightens its interactions with associated proteins, dramatically lowering plasticity and becoming rigid. This process may be the underlying biophysical basis for the vast changes in transcriptional profiles that pluripotent cells undergo during differentiation.

Epigenetic effectors of chromatin plasticity

We have described chromatin plasticity as the underlying biophysical basis for the transcriptional profile and subsequent gene-expression pattern that is unique to pluripotent cells. We have discussed how profound changes in chromatin plasticity alter this transcriptional profile and characterize embryonic development, from pluripotent to differentiated cells. We now elaborate on the molecular mechanisms, epigenetic and others, that regulate chromatin plasticity in pluripotent and differentiating cells (Figure 3).

DNA methylation

The only epigenetic mark on DNA itself is the covalent methylation of cytosine residues, predominately, but not exclusively, in CpG dinucleotides. This modification is first erased by pan-genomic demethylation in the zygote and then reintroduced by de novo Dnmts (DNA methyltransferases) Dnmt3a and Dnmt3b in early embryonic development, between the morula and blastocyst stages, or during growth in culture of ICM-derived ESCs. DNA methylation is then maintained in all cells throughout the life cycle by Dnmt1 [1,2,32–34]. Although somatic cells require DNA methylation for cellular functions and normal growth, and although DNA methylation is required for ESC differentiation, undifferentiated ESCs are refractory to depletion of DNA methylation, and retain self-renewal and pluripotency in the absence of Dnmts [35,36].

Viewed in concert with histone modifications, DNA methylation is considered to be a major transcriptional regulator [1]. Promoter methylation is associated with gene repression [37]. Promoters of tissue-specific genes are methylated in pluripotent ESCs, but not in multipotent adult stem cells [38]. Globally hypomethylated Dnmt-triple-knockout ESCs lacking Dnmt1/3a/3b express unusually high levels of tissue-specific genes [39]. In contrast, promoters of pluripotency-related genes remain hypomethylated in early development and in ESCs; they are methylated during differentiation and are actively demethylated during reprogramming into iPSCs [33]. The active DNA demethylation mechanism has yet to be deciphered, but it seems to be AID
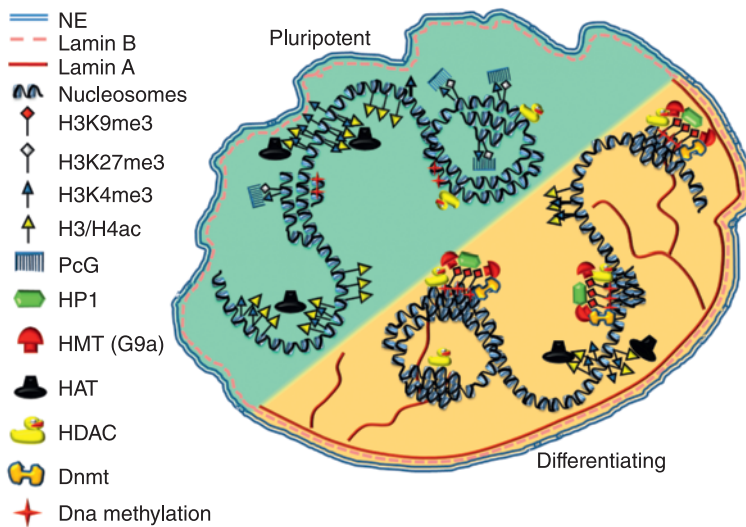
Figure 3. The pluripotent compared with the differentiating nucleus
Comparative view of nuclear structure and chromatin plasticity in an undifferentiated pluripotent cell (green) undergoing differentiation (orange). Changes illustrated include the nuclear envelope and lamina that round out, acquiring form and rigidity upon the expression of lamin A (red filament); heterochromatin foci become numerous, smaller and more condensed; heterochromatin propagation is facilitated by G9a-mediated H3K9me3 (red squares flanked by red mushrooms) that recruits HP1 (green hexagon), HDACs (yellow duck) and Dnmts (yellow dumbbells, DNA methylation is represented by red stars); the prevalent active chromatin marks, histone acetylation (yellow arrowheads) by histone acetyltransferases (HAT, black hat) and H3K4me3 (blue arrowheads), are significantly reduced; chromatin that is 'bivalently' marked (blue arrowheads neighbouring white squares) and bound by polycomb group proteins (blue combs) becomes either active or repressed.

(activation-induced cytidine deaminase)-dependent [40]. Furthermore, pluripotency-related gene promoters were found to have UMR (unmethylated region) sequence motifs that are methylated upon differentiation and demethylated during reprogramming in a non-CpG-island context [41].

A study in mouse and fruitfly suggested more than a decade ago that 15–25% of DNA methylation in ESCs is in a non-CpG context, at least 5-fold higher than in various somatic tissues. The *de novo* Dnmt3a was implicated in this [42]. Recently, a genome-wide single-base-resolution DNA methylation study compared DNA methylation profiles between human ESCs, human fibroblasts and iPSCs derived from these fibroblasts [43]. Evidently, the high levels of non-CpG methylation in ESCs are not maintained in differentiated cells and reappear upon reprogramming into iPSCs, all in correlation with Dnmt3a/b expression levels that show the same pattern. This pluripotency-related non-CpG methylation pattern was found mostly in gene bodies, particularly on the template strand in exons, it is tightly associated with gene expression and possibly linked to RNA polymerase activity. Non-CpG

methylation appears to be a DNA 'switch', toggling between pluripotency/differentiation states and governing the expression of gene families underlying them. Could this switch be linked to rapid changes in chromatin plasticity?

We set out to inspect the relationship between global DNA methylation levels and chromatin plasticity in ESCs (S. Melcer, H. Hezroni, E. Rand, A. Skoultchi, M. Bustin and E. Meshorer, unpublished work). Using DNA-demethylating agents and mutant mouse ESC lines, lacking one or more of the Dnmt genes, we compared chromatin protein dynamics between ESCs with high, low or no DNA methylation. Although we confirmed that both *de novo* and maintenance DNA methylation are independently essential for proper ESC differentiation, we found they have an insignificant effect on chromatin plasticity. It should also be taken into account that *in vitro* cell proliferation has been suspected of causing aberrant DNA-methylation patterns [44], possibly masking molecular mechanisms that govern DNA-methylation-linked chromatin plasticity. Whether it also contributes to chromatin protein dynamics remains obscure.

There is a growing consensus as to the link between DNA methylation and histone modifications, especially histone methylation [1]. DNA methylation was shown to be only the first of several steps regulating gene expression of tissue-specific promoters during differentiation [38], supporting a model in which DNA methylation is a relatively minor regulator of chromatin plasticity compared with the histone-modification network and chromatin remodellers, which are discussed in the next section.

The 'histone code' and chromatin remodelling

Core histones have N-terminal tail domains that are subject to a wide variety of covalent (albeit reversible) post-translational modifications, such as acetylation, methylation, phosphorylation, ubiquitination, SUMOylation and poly(ADP-ribosyl)ation, each regulated by a family of modifying and de-modifying enzymes [3]. It is widely accepted that histone modifications dictate specific chromatin states [45]. Also, some modifications depend on the existence of others; some modifications inhibit others and some are mutually affected/regulated [46]. The result is a high variability of the otherwise identical nucleosome cores that seems to form an epigenetic 'code' affecting gene expression, either via certain modifications or a combination of several different modifications on a group of nucleosomes [47]. This 'code' is believed to be specifically 'read' by enzymes involved in transcription regulation or chromatin remodelling [48,49] and is kept in a heritable cellular 'memory' [46]. The histone code consists mainly of expressive marks, including pan-acetylation of histones H3 and H4 (H3ac, H4ac), tri-methylation of Lys^4 in histone H3 (H3K4me3), H3K36me2/3, H3K9ac H3K14ac, H4K16ac, H2BK5me1 and more; and repressive or heterochromatic marks, including H3K9me1/2/3, H3K27me2/3, H4K20me3 and H2AK119ub [3,50].

Furthermore, as indicated above, histone modifications have an intricate cross-talk network not only among themselves, but also with other epigenetic factors, such as DNA methylation [1,38]. There is evidence, for example, that the H3K4me2 mark protects DNA from methylation [51]. This cross-talk is mediated by a variety of chromatin-remodelling proteins and complexes (some of which are discussed below), that either change higher-order chromatin structure or recruit other chromatin remodellers, histone modifiers, Dnmts and demethylases and more. This is also true for heterochromatin. Tri-methylated H3K9, for example, is a binding site for HP1 which recruits Dnmts and can facilitate the spreading of silenced chromatin via DNA methylation [52].

There is accumulating evidence that the histone code is far more complex than first perceived, includes a multitude of signals that should be read not separately, but in concert, and orchestrates a vast number of biological processes that greatly exceed the scope of gene expression regulation [53]. Various high-throughput genome-wide analyses, such as ChIP (chromatin immunoprecipitation) followed by microarray analyses (ChIP-on-chip) and ChIP followed by high-throughput sequencing (ChIP-seq), greatly advance our understanding of chromatin states and their regulation by histone-modification profiles in different cells and throughout development, supplying new tools to dissect such cellular states as pluripotency [50,54].

Among their many unique characteristics, pluripotent and multipotent cells have distinct histone 'codes' underlying their full or partial 'stemness'-related transcriptional profiles, described above. Differentiation genes are bivalently marked in ESCs with both active and repressive marks [55]. During differentiation, the repressive marks on relevant genes are gradually removed, leaving the expression marks exposed, resulting in rapid and efficient expression [38].

Polycomb proteins are found in genomic regions rich with developmental regulators, repressing them, thereby preventing differentiation and maintaining pluripotency in ESCs. Polycomb complexes contain the enzymes that methylate Lys^{27} of histone H3 (i.e. Ezh2) and therefore these regions are characterized by H3K27me3 marks [56,57]. In addition to H3K27me3, H3K4me3 is also frequently found in these regions, thus forming a 'bivalent' mark, with histones simultaneously marked for both transcription and repression [55]. During ESC differentiation, some of these bivalent domains are resolved, according to the relevant cell type [58]. As mentioned above, protein-coding chromatin regions in ESCs predominantly have nucleosomes with marks characteristic of transcription start sites, namely H3K4me3, H3K9ac, H3K14ac and RNAPIIp, albeit only part of these marked regions additionally carry the H3K36me3 hallmark for elongation [17]. Such post-initiation regulation is also found in differentiated cells, but in a more cell-type-specific manner.

In line with repression of pervasive transcription during ESC differentiation [18], there is a gradual increase in repressive histone marks such as H3K9me3 [5] and H3K27me3 [30], and a concomitant decrease in permissive marks, including H3K9ac [59] and H3ac [29].

A specific interest is taken in the pluripotency master regulator, Oct4. In ESCs, the *Oct4* locus is enriched with active marks, such as H3ac, H4ac, H3S10p and H3K4me1/2/3. Neuronal differentiation of ESCs results in a gradual removal of these marks, H3K4me2 being the last mark to persist until the fully differentiated state, albeit at low levels [30].

Nanog and Tcl1 are two additional important self-renewal factors in ESCs, downstream targets of Oct4. Oct4 up-regulates H3K9 demethylases, Jmjd1a and Jmjd2c, actively removing H3K9me2 and H3K9me3 repressive marks from Tcl1 and Nanog promoters respectively, thus maintaining self-renewal in ESCs. Upon differentiation and Oct4 repression, down-regulation of Jmjd1a and Jmjd2c leads to accumulation of repressive H3K9 methylation marks and subsequent shut down of the Tcl1/Nanog self-renewal mechanism [60].

In our recent attempt to delineate the mechanisms that underlay chromatin plasticity regulation in ESCs (S. Melcer, H. Hezroni, E. Rand, A. Skoultchi, M. Bustin and E. Meshorer, unpublished work), we examined the effects of both histone acetylation and methylation on chromatin protein dynamics.

We found, using FRAP assays, that increasing histone acetylation levels by inhibition of HDAC (histone deacetylase) activity correlated with increased chromatin protein dynamics in ESCs, in ESC-derived NPCs and in somatic fibroblasts. Additionally, when comparing spontaneous differentiation levels, hyperacetylated ESCs retained their pluripotency, and spontaneous differentiation was inhibited more than 2-fold over normally acetylated ESCs. In contrast, HDAC inhibitors augmented neuronal differentiation in NPCs, suggesting that an epigenetic barrier exists early during ESC differentiation after which differentiation cannot be prevented by increasing histone acetylation. Thus histone acetylation affects chromatin plasticity in differentiated cells, in pluripotent cells and in multipotent lineage-committed cells, subsequently affecting their propensity to differentiate, albeit in a differentiation-stage-dependent manner.

Histone methylation also plays important roles in regulating chromatin plasticity in ESCs. Using a mutant ESC line lacking G9a, the H3K9 monomethylase [61], we found that although undifferentiated knockout and wild-type cells displayed similar H1–GFP (green fluorescent protein) dynamics, chromatin in G9a$^{-/-}$ ESC-derived NPCs was hyperdynamic compared with wild-type NPCs. This effect was prominent in heterochromatin foci and hardly seen in euchromatin. Furthermore, G9a$^{-/-}$ ESCs failed to complete neuronal differentiation. Adding G9a back into the mutant ESCs fully restored neuronal differentiation capacity as well as wild-type chromatin protein dynamics throughout differentiation. Thus histone methylation in heterochromatin is at least partly responsible for chromatin restriction in differentiating ESCs, which is evidently required for proper differentiation. It should be noted that the effects observed in context with G9a were not observed in ESCs lacking Suv39h1/2, the H3K9 di/tri-methylases; this is in line with earlier studies showing that H3K9me2/3 is a redundant mark and, when lost, it is replaced by H3K9me1 (by G9a) and H3K27me3 [52,62].

These findings demonstrate further the function of chromatin plasticity in maintaining pluripotency, commencing and completing differentiation, and it appears that a unique pattern of certain histone modifications is the basis of this crucial chromatin state.

Having discussed this wide variety of active, repressive and 'bivalent' chromatin marks, it should be stressed that these marks do not affect chromatin plasticity itself, but rather post a mark on designated chromatin regions to be modelled or remodelled into the corresponding architecture. Chromatin marks, on both histones and DNA, act as binding or recognition sites for various chromatin-remodelling proteins; these are characterized by ATPase domains, chromodomains, bromodomains and different binding motifs that recruit other participants in chromatin marking or structuring [3,52,53]. For example, G9a-mediated methylation recruits HP1 via its chromodomain, H3K27me2/3 recruits polycomb proteins, and H3K4me1 recruits both HATs (histone acetyltransferases), via their bromodomain, and the Jmjd2a histone demethylase [3,53]. This accumulated evidence elucidates the mechanistic principle underlying the co-localization of H3K9me3, HP1α and resulting heterochromatin regions in ESCs, their reformation during differentiation and effects on chromatin plasticity [5].

Another good example of this histone-code–chromatin-remodelling network is the H3K4me3-recruited Chd1 (chromodomain-helicase-DNA-binding protein 1), which seems to regulate both chromatin plasticity and pluripotency in ESCs [63]. In Chd1-deficient ESCs, heterochromatin is more prevalent, and chromatin protein dynamics are reduced in euchromatic regions, implicating Chd1 as a chromatin remodeller that participates in maintaining an open-chromatin formation in undifferentiated ESCs. Without Chd1, ESCs are not truly pluripotent; they are prone to neural-lineage (ectoderm) differentiation and do not give rise to primitive endoderm [63]. Analysing Chd1 function by the aforementioned standards, it is likely that Chd1 'reads' H3K4me3-marked regions, most probably in conjunction with other pluripotent-related chromatin marks yet unknown, and aptly promotes open-chromatin formation in these regions.

This combination of active and repressive histone marks accompanied by antagonist chromatin-associated proteins provides a reasonable explanation for pluripotent-related chromatin plasticity. A spectrum of chromatin-associated proteins with opposite architectural functions is recruited by apparently incoherent signals to a wide variety of regulatory junctions in the genome. This results in multiple gene loci that are primed for transcription, rendering chromatin highly plastic and hyperdynamic, but without the subsequent multitude of expression products one would expect from over-transcriptional activity.

Lamin A

Lamins are type V IFs (intermediate filaments) that comprise the nuclear lamina, a proteinaceous meshwork underlining the INM (inner nuclear membrane) of the nuclear envelope. Lamins have a wide network of

interactions with other lamins, INM proteins, transcription factors, chromatin and DNA and they are known to have a role in a large variety of nuclear processes [64]. Lamins are divided into two groups: A- and B-type. A-type lamins, predominantly lamin A and lamin C, are expressed from the LMNA gene, mutations in which are linked directly to a growing list of diseases termed 'laminopathies' [64,65].

Several clues point to a connection between lamin A and chromatin plasticity regulation in differentiating cells. First, whereas B-type lamins are expressed in all cells throughout development, A-type lamins are not detected in ESCs, but only in early differentiating cells, at the stage when chromatin proteins become immobile [64,66]. Differentiating and somatic cells exhibit defined nuclear envelopes, smooth and round, with a highly stable nuclear lamina characterized by tight lamin interactions and low turnover rates, whereas ESCs have a nuclear lamina that seems to be more 'fluid' both in structure (relatively amorphous) and dynamics (relatively loose binding and fast turnover) [6,29]. These differences in lamina structure apparently have a mechanical effect on the nuclear envelope; differentiating cell nuclei are more rigid and less prone to deformation than ESC nuclei that exhibit not only high chromatin plasticity, but also high physical plasticity [67]. Secondly, lamin A was shown to bind DNA and chromatin [68–70], and specific conserved sequences in *Drosophila* and *Caenorhabditis elegans* were shown to specifically bind histone H2A [71]. Various $Lmna^{-/-}$-mice-derived cells have aberrant heterochromatin and nucleoli organization [72]. Thirdly, many studies link lamin A with regulation of transcription factors, gene expression and chromatin organization [64,70], and a study of HGPS (Hutchinson–Gilford progeria syndrome) implicated mutant lamin A in causing epigenetic alterations (down-regulation of the Ezh2 methyltransferase, loss of H3K27me3 on the inactive X chromosome, reduction in H3K9me3 and HP1α binding and increase in H4K20me3) and subsequent chromatin structure aberrations (loss of heterochromatin) [73].

Considering the above evidence, lamin A may serve as an additional player controlling chromatin plasticity by restricting chromatin dynamics upon early differentiation. Combining additive and subtractive approaches, we examined chromatin plasticity both in wild-type mouse ESCs ectopically expressing lamin A and in lamin A-deficient ESCs and MEFs derived from $Lmna^{-/-}$ mice (S. Melcer, H. Hezroni, E. Rand, A. Skoultchi, M. Bustin and E. Meshorer, unpublished work). We found that lamin A significantly restricts chromatin protein dynamics, exclusively in heterochromatin regions, when ectopically expressed in wild-type ESCs. Surprisingly, we witnessed no such effect when comparing chromatin protein dynamics between wild-type and $Lmna^{-/-}$ MEFs, suggesting that some redundancy exists in this form of chromatin plasticity regulation and that overlapping mechanisms may be activated during differentiation. Supporting this possibility is our observation that $Lmna^{-/-}$ ESCs differentiate fully and properly into NPCs and post-mitotic neurons, a process that was compromised when other chromatin plasticity regulators were perturbed.

Interestingly, the ESC nuclear periphery has been shown to associate with both repressive and active chromatin marks, with both gene-rich and gene-poor regions, all in context with lamin B and in the absence of the yet-to-be-expressed lamin A/C [74]. The repressive mark H3K27me3, which is adjacent to the nuclear envelope in undifferentiated ESCs, is reduced and is redistributed upon differentiation and concomitant expression of lamin A. Although the connection between lamin A expression and H3K27me3 de-localization might be circumstantial, it does join evidence regarding lamina plasticity loss and overall nuclear stiffening, including chromatin structure [29,67], raising the possibility that certain gene-regulation mechanisms react to acute changes in plasticity.

Lamin A is generally associated with nuclear structural and functional integrity and takes part in many nuclear processes. As such, it is a difficult task to specifically link it with just one of its many roles in proper cell development and function, including chromatin plasticity, in the light of pleiotropic effects. Nevertheless, there is a considerable variety of evidence from different sources, independently implicating lamin A, specifically, as a chromatin plasticity regulator. Our work suggests that it would be a challenging task to further fine-tune the elucidation of this regulatory function.

Conclusions and future perspectives

As we have seen, chromatin in ESCs is distinct from that of differentiated and somatic cells in many respects. It seems that the unique properties featured in ESC chromatin mostly contribute to its plastic state regulating ESC pluripotency, self-renewal capacity and differentiation potential. Since ESCs hold great promise for degenerative diseases and cell-based therapy, it is essential to understand the basic mechanisms that make ESCs what they are in order to manipulate ESCs intelligently and safely and facilitate their use in the clinic. Since chromatin is the basic scaffold for genetic stability, genetic and epigenetic inheritance, gene expression and regulation and cell-cycle progression, understanding chromatin plasticity in ESCs is key to understanding pluripotency and ESC function. Recent advances in high-throughput technologies, such as massively parallel sequencing and multi-dimensional proteomics, will enable rapid profiling of epigenetic states in ESCs and efficient identification of molecules that regulate chromatin in ESCs. Once the basic biology is understood, it will lay the ground to take the extra necessary step towards the clinic.

Summary

- *ESCs are pluripotent and self-renew indefinitely.*
- *Undifferentiated ESCs display a global promiscuous transcriptional programme which is restricted gradually upon differentiation.*

- *Chromatin in pluripotent cells is more 'plastic' or 'open', with decondensed heterochromatin architecture and chromatin protein hyperdynamics.*
- *ESC differentiation is characterized by substantial changes, including, (i) reduction in chromatin plasticity, exhibited as structural condensation and restriction of chromatin protein dynamics; (ii) significant changes in chromatin modifications, including histone marks and DNA methylation; and (iii) changes in nuclear architecture.*
- *Regulation of epigenetic patterns and chromatin plasticity are essential for pluripotency and self-renewal.*

References

1. Cedar, H. and Bergman, Y. (2009) Linking DNA methylation and histone modification: patterns and paradigms. Nat. Rev. Genet. **10**, 295–304
2. Delcuve, G.P., Rastegar, M. and Davie, J.R. (2009) Epigenetic control. J. Cell. Physiol. **219**, 243–250
3. Campos, E.I. and Reinberg, D. (2009) Histones: annotating chromatin. Annu. Rev. Genet. **43**, 559–599
4. Misteli, T. (2007) Beyond the sequence: cellular organization of genome function. Cell **128**, 787–800
5. Meshorer, E., Yellajoshula, D., George, E., Scambler, P.J., Brown, D.T. and Misteli, T. (2006) Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. Dev. Cell **10**, 105–116
6. Meshorer, E. and Misteli, T. (2006) Chromatin in pluripotent embryonic stem cells and differentiation. Nat. Rev. Mol. Cell Biol. **7**, 540–546
7. Collas, P. (2009) Epigenetic states in stem cells. Biochim. Biophys. Acta **1790**, 900–905
8. Hochedlinger, K. and Plath, K. (2009) Epigenetic reprogramming and induced pluripotency. Development **136**, 509–523
9. Waddington, C. (1942) The epigenotype. Endeavour **1**, 18–20
10. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell **126**, 663–676
11. Amabile, G. and Meissner, A. (2009) Induced pluripotent stem cells: current progress and potential for regenerative medicine. Trends Mol. Med. **15**, 59–68
12. Loh, Y.H., Ng, J.H. and Ng, H.H. (2008) Molecular framework underlying pluripotency. Cell Cycle **7**, 885–891
13. Chen, L. and Daley, G.Q. (2008) Molecular basis of pluripotency. Hum. Mol. Genet. **17**, R23–27
14. Surani, M.A., Hayashi, K. and Hajkova, P. (2007) Genetic and epigenetic regulators of pluripotency. Cell **128**, 747–762
15. Giadrossi, S., Dvorkina, M. and Fisher, A.G. (2007) Chromatin organization and differentiation in embryonic stem cell models. Curr. Opin. Genet. Dev. **17**, 132–138
16. Boyer, L.A., Lee, T.I., Cole, M.F., Johnstone, S.E., Levine, S.S., Zucker, J.P., Guenther, M.G., Kumar, R.M., Murray, H.L., Jenner, R.G. et al. (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. Cell **122**, 947–956
17. Guenther, M.G., Levine, S.S., Boyer, L.A., Jaenisch, R. and Young, R.A. (2007) A chromatin landmark and transcription initiation at most promoters in human cells. Cell **130**, 77–88
18. Efroni, S., Duttagupta, R., Cheng, J., Dehghani, H., Hoeppner, D.J., Dash, C., Bazett-Jones, D.P., Le Grice, S., McKay, R.D., Buetow, K.H. et al. (2008) Global transcription in pluripotent embryonic stem cells. Cell Stem Cell **2**, 437–447
19. Ram, E.V. and Meshorer, E. (2009) Transcriptional competence in pluripotency. Genes Dev. **23**, 2793–2798

20. Golan-Mashiach, M., Dazard, J.E., Gerecht-Nir, S., Amariglio, N., Fisher, T., Jacob-Hirsch, J., Bielorai, B., Osenberg, S., Barad, O., Getz, G. et al. (2005) Design principle of gene expression used by human stem cells: implication for pluripotency. FASEB J. **19**, 147–149

21. Hu, M., Krause, D., Greaves, M., Sharkis, S., Dexter, M., Heyworth, C. and Enver, T. (1997) Multilineage gene expression precedes commitment in the hemopoietic system. Genes Dev. **11**, 774–785

22. Terskikh, A.V., Miyamoto, T., Chang, C., Diatchenko, L. and Weissman, I.L. (2003) Gene expression analysis of purified hematopoietic stem cells and committed progenitors. Blood **102**, 94–101

23. Efroni, S., Melcer, S., Nissim-Rafinia, M. and Meshorer, E. (2009) Stem cells do play with dice: a statistical physics view of transcription. Cell Cycle **8**, 43–48

24. Zipori, D. (2004) The nature of stem cells: state rather than entity. Nat. Rev. Genet. **5**, 873–878

25. Mikkelsen, T.S., Hanna, J., Zhang, X., Ku, M., Wernig, M., Schorderet, P., Bernstein, B.E., Jaenisch, R., Lander, E.S. and Meissner, A. (2008) Dissecting direct reprogramming through integrative genomic analysis. Nature **454**, 49–55

26. Toyooka, Y., Shimosato, D., Murakami, K., Takahashi, K. and Niwa, H. (2008) Identification and characterization of subpopulations in undifferentiated ES cell culture. Development **135**, 909–918

27. Furusawa, C. and Kaneko, K. (2009) Chaotic expression dynamics implies pluripotency: when theory and experiment meet. Biol. Direct **4**, 17

28. Wernig, M., Lengner, C.J., Hanna, J., Lodato, M.A., Steine, E., Foreman, R., Staerk, J., Markoulaki, S. and Jaenisch, R. (2008) A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. Nat. Biotechnol. **26**, 916–924

29. Bhattacharya, D., Talwar, S., Mazumder, A. and Shivashankar, G.V. (2009) Spatio-temporal plasticity in chromatin organization in mouse cell differentiation and during *Drosophila* embryogenesis. Biophys. J. **96**, 3832–3839

30. Aoto, T., Saitoh, N., Ichimura, T., Niwa, H. and Nakao, M. (2006) Nuclear and chromatin reorganization in the MHC–Oct3/4 locus at developmental phases of embryonic stem cell differentiation. Dev. Biol. **298**, 354–367

31. Reference deleted

32. Pannetier, M. and Feil, R. (2007) Epigenetic stability of embryonic stem cells and developmental potential. Trends Biotechnol. **25**, 556–562

33. Farthing, C.R., Ficz, G., Ng, R.K., Chan, C.F., Andrews, S., Dean, W., Hemberger, M. and Reik, W. (2008) Global mapping of DNA methylation in mouse promoters reveals epigenetic reprogramming of pluripotency genes. PLoS Genet. **4**, e1000116

34. Hemberger, M., Dean, W. and Reik, W. (2009) Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. Nat. Rev. Mol. Cell Biol. **10**, 526–537

35. Jackson, M., Krassowska, A., Gilbert, N., Chevassut, T., Forrester, L., Ansell, J. and Ramsahoye, B. (2004) Severe global DNA hypomethylation blocks differentiation and induces histone hyperacetylation in embryonic stem cells. Mol. Cell. Biol. **24**, 8862–8871

36. Tsumura, A., Hayakawa, T., Kumaki, Y., Takebayashi, S., Sakaue, M., Matsuoka, C., Shimotohno, K., Ishikawa, F., Li, E., Ueda, H.R. et al. (2006) Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. Genes Cells **11**, 805–814

37. Miranda, T.B. and Jones, P.A. (2007) DNA methylation: the nuts and bolts of repression. J. Cell. Physiol. **213**, 384–390

38. Aranda, P., Agirre, X., Ballestar, E., Andreu, E.J., Roman-Gomez, J., Prieto, I., Martin-Subero, J.I., Cigudosa, J.C., Siebert, R., Esteller, M. and Prosper, F. (2009) Epigenetic signatures associated with different levels of differentiation potential in human stem cells. PLoS ONE **4**, e7809

39. Fouse, S.D., Shen, Y., Pellegrini, M., Cole, S., Meissner, A., Van Neste, L., Jaenisch, R. and Fan, G. (2008) Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. Cell Stem Cell **2**, 160–169

40. Bhutani, N., Brady, J.J., Damian, M., Sacco, A., Corbel, S.Y. and Blau, H.M. (2010) Reprogramming towards pluripotency requires AID-dependent DNA demethylation. Nature **463**, 1042–1047

41. Straussman, R., Nejman, D., Roberts, D., Steinfeld, I., Blum, B., Benvenisty, N., Simon, I., Yakhini, Z. and Cedar, H. (2009) Developmental programming of CpG island methylation profiles in the human genome. Nat. Struct. Mol. Biol. **16**, 564–571

42. Ramsahoye, B.H., Biniszkiewicz, D., Lyko, F., Clark, V., Bird, A.P. and Jaenisch, R. (2000) Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. Proc. Natl. Acad. Sci. U.S.A. **97**, 5237–5242

43. Lister, R., Pelizzola, M., Dowen, R.H., Hawkins, R.D., Hon, G., Tonti-Filippini, J., Nery, J.R., Lee, L., Ye, Z., Ngo, Q.M. et al. (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature **462**, 315–322

44. Meissner, A., Mikkelsen, T.S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., Zhang, X., Bernstein, B.E., Nusbaum, C., Jaffe, D.B. et al. (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature **454**, 766–770

45. Turner, B.M. (1993) Decoding the nucleosome. Cell **75**, 5–8

46. Turner, B.M. (2002) Cellular memory and the histone code. Cell **111**, 285–291

47. Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. Nature **403**, 41–45

48. Taverna, S.D., Li, H., Ruthenburg, A.J., Allis, C.D. and Patel, D.J. (2007) How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat. Struct. Mol. Biol. **14**, 1025–1040

49. Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. Science **293**, 1074–1080

50. Schones, D.E. and Zhao, K. (2008) Genome-wide approaches to studying chromatin modifications. Nat. Rev. Genet. **9**, 179–191

51. Weber, M., Hellmann, I., Stadler, M.B., Ramos, L., Paabo, S., Rebhan, M. and Schubeler, D. (2007) Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat. Genet. **39**, 457–466

52. Lund, A.H. and van Lohuizen, M. (2004) Epigenetics and cancer. Genes Dev. **18**, 2315–2335

53. Sims, R.J., 3rd and Reinberg, D. (2008) Is there a code embedded in proteins that is based on post-translational modifications? Nat. Rev. Mol. Cell Biol. **9**, 815–820

54. Hon, G.C., Hawkins, R.D. and Ren, B. (2009) Predictive chromatin signatures in the mammalian genome. Hum. Mol. Genet. **18**, R195–R201

55. Bernstein, B.E., Mikkelsen, T., Xie, X., Kamal, M., Huebert, D., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K. et al. (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell **125**, 315–326

56. Lee, T.I., Jenner, R.G., Boyer, L.A., Guenther, M.G., Levine, S.S., Kumar, R.M., Chevalier, B., Johnstone, S.E., Cole, M.F., Isono, K. et al. (2006) Control of developmental regulators by polycomb in human embryonic stem cells. Cell **125**, 301–313

57. Boyer, L.A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L.A., Lee, T.I., Levine, S.S., Wernig, M., Tajonar, A., Ray, M.K. et al. (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature **441**, 349–353

58. Mikkelsen, T.S., Ku, M., Jaffe, D.B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W., Kim, T.K., Koche, R.P. et al. (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature **448**, 553–560

59. Krejci, J., Uhlirova, R., Galiova, G., Kozubek, S., Smigova, J. and Bartova, E. (2009) Genome-wide reduction in H3K9 acetylation during human embryonic stem cell differentiation. J. Cell. Physiol. **219**, 677–687

60. Loh, Y.H., Zhang, W., Chen, X., George, J. and Ng, H.H. (2007) Jmjd1a and Jmjd2c histone H3 Lys[9] demethylases regulate self-renewal in embryonic stem cells. Genes Dev. **21**, 2545–2557

61. Tachibana, M., Sugimoto, K., Nozaki, M., Ueda, J., Ohta, T., Ohki, M., Fukuda, M., Takeda, N., Niida, H., Kato, H. and Shinkai, Y. (2002) G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes Dev. **16**, 1779–1791

62. Peters, A.H., Kubicek, S., Mechtler, K., O'Sullivan, R.J., Derijck, A.A., Perez-Burgos, L., Kohlmaier, A., Opravil, S., Tachibana, M., Shinkai, Y. et al. (2003) Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol. Cell **12**, 1577–1589

63. Gaspar-Maia, A., Alajem, A., Polesso, F., Sridharan, R., Mason, M.J., Heidersbach, A., Ramalho-Santos, J., McManus, M.T., Plath, K., Meshorer, E. and Ramalho-Santos, M. (2009) Chd1 regulates open chromatin and pluripotency of embryonic stem cells. Nature **460**, 863–868

64. Dechat, T., Pfleghaar, K., Sengupta, K., Shimi, T., Shumaker, D.K., Solimando, L. and Goldman, R.D. (2008) Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev. **22**, 832–853

65. Worman, H.J., Fong, L.G., Muchir, A. and Young, S.G. (2009) Laminopathies and the long strange trip from basic cell biology to therapy. J. Clin. Invest. **119**, 1825–1836

66. Constantinescu, D., Gray, H.L., Sammak, P.J., Schatten, G.P. and Csoka, A.B. (2006) Lamin A/C expression is a marker of mouse and human embryonic stem cell differentiation. Stem Cells **24**, 177–185

67. Pajerowski, J.D., Dahl, K.N., Zhong, F.L., Sammak, P.J. and Discher, D.E. (2007) Physical plasticity of the nucleus in stem cell differentiation. Proc. Natl. Acad. Sci. U.S.A. **104**, 15619–15624

68. Taniura, H., Glass, C. and Gerace, L. (1995) A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. J. Cell Biol. **131**, 33–44

69. Glass, J. and Gerace, L. (1990) Lamins A and C bind and assemble at the surface of mitotic chromosomes. J. Cell Biol. **111**, 1047–1057

70. Andres, V. and Gonzalez, J.M. (2009) Role of A-type lamins in signaling, transcription, and chromatin organization. J. Cell Biol. **187**, 945–957

71. Mattout, A., Goldberg, M., Tzur, Y., Margalit, A. and Gruenbaum, Y. (2007) Specific and conserved sequences in *D. melanogaster* and *C. elegans* lamins and histone H2A mediate the attachment of lamins to chromosomes. J. Cell Sci. **120**, 77–85

72. Nikolova, V., Leimena, C., McMahon, A.C., Tan, J.C., Chandar, S., Jogia, D., Kesteven, S.H., Michalicek, J., Otway, R., Verheyen, F. et al. (2004) Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C-deficient mice. J. Clin. Invest. **113**, 357–369

73. Shumaker, D.K., Dechat, T., Kohlmaier, A., Adam, S.A., Bozovsky, M.R., Erdos, M.R., Eriksson, M., Goldman, A.E., Khuon, S., Collins, F.S. et al. (2006) Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. Proc. Natl. Acad. Sci. U.S.A. **103**, 8703–8708

74. Luo, L., Gassman, K.L., Petell, L.M., Wilson, C.L., Bewersdorf, J. and Shopland, L.S. (2009) The nuclear periphery of embryonic stem cells is a transcriptionally permissive and repressive compartment. J. Cell Sci. **122**, 3729–3737