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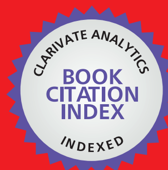

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Chapter 10

Epigenetic Reprogramming in Stem Cells

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http://dx.doi.org/10.5772/56327

1. Introduction

This chapter will be focused on epigenetic mechanisms known to affect self-renewal and developmental potency of embryonic-like stem cells, and germ cells which mimic similar epigenetic signatures as pluripotent stem cells. Examples of epigenetic regulation have proven crucial for defining the stem cell state. In particular, a wealth of knowledge regarding stem cell-specific epigenetic modifications has occurred over the past decade with discoveries that include describing unique stem cell-specific chromosome structure, DNA and histone modifications and noncoding RNAs. The impact of these findings and the better understanding of epigenetic regulation in pluripotent stem cells provides a foundation for discovering mechanisms which regulate human development and differentiation in addition to those that can facilitate cellular reprogramming.

In eukaryotes, chromosomes consist of repeating chromatin units called nucleosomes, which encompass segments of DNA (~147 bp) wound around a central core of eight histone (**H**) proteins (two each of core histone proteins H2A, H2B, H3, and H4). These units are separated by a linker DNA associated with histone protein, H1. Post-translational modifications of these histone residues regulate gene expression [1, 2]. The types of modification of specific amino acids within these proteins include acetylation (*Ac*), mono- di- or tri-methylation (*Me1, 2, 3*), and ubiquitylation (**Ub**). Although these mechanism in general appear to work independently, recent evidence has demonstrated that crosstalk does exist between some of these modifications [1, 3]. Chromatin can be distinguished based on its anatomical structure as either heterochromatin or euchromatin. Heterochromatin is characterized by tightly packed nucleosomes that occur at centromeres, telomeres, and areas of repetitive DNA and is associated with low gene transcription while euchromatin constitute less compacted areas of chromatin and associated with active gene expression. In general, these areas of chromatin are non-randomly distributed within the nucleus and cell-type and cell-cycle dependent [4, 5].

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In mammals, heterochromatin is associated with high levels of some methylation marks, including lysine (**K**)-9, K-27 and K-20 on histone (**H**) -3 (H3K9me3, H3K27me3, H3K20me3), low levels of acetylation, and its associated proteins including heterochromatin protein 1 (**HP1**) [6]. In contrast, euchromatin is associated with high levels of acetylation and methylation marks, such as K-4, K-36, and K79 on Histone 3 (H3K4, H3K36 and H3K7)(reviewed by [1]). These modifications occur through the activity of the following enzymes, histone acetyl transferases (**HATs**), histone deacetylases (**HDACs**), histone methyltransferases (**HMT**) and histone demethylases (**HDMT**). Further chromatin regulation is also possible through modifications of the histone tails which are suspected to produce a 'histone code' that initiate higher order chromatin folding [7].

2. Epigenetic control in pluripotent stem cells

2.1. Changes in chromatin ultrastructure in pluripotent stem cells

With the discovery of culturing embryonic stem cells (ESCs), several groups have been able to show the progression of global changes in the chromatin architecture of these cells. Through these studies, it has been shown that undifferentiated pluripotent stem cells contain less heterochromatic regions and express less chromatin structural proteins. Moreover, binding of these proteins (i.e. HP1α, lamin B) to heterochromatic regions is weaker compared to lineage-committed cells [8]. Additionally, pluripotent transcription factors and chromatin remodeling proteins are overexpressed in ESCs compared to more differentiated progenitor cells [9]. One study further showed that chromatin remodeler Chd1 knockdown results with heterochromatin accumulation and skewed differentiation in mouse ESCs, which suggests functional relevancy to the 'open' chromatin structure [10]. Together, these studies show that pluripotent ESCs has an open and hyperdynamic chromatin structure which transforms into a more compact, repressive-like, chromatin state during differentiation.

As ESC chromatin is more transcriptionally permissive, it is also more sensitive to nuclease activity. This may also be in part due to differences that are seen in the chromatin localization in the nucleus. For instance, one study using the DamID (DNA adenine methyltransferase identification) technique showed that pluripotency genes, including Oct4 and Nanog, move to the nuclear lamina and are silenced while lineage-specific genes disassociate from the lamina and are expressed. This was specifically shown during the differentiation of mouse ESCs into terminal astrocytes which demonstrated cell type-specific relocations of these areas during differentiation [11]. These areas near the nuclear periphery were called Lamina Associated Domains (**LADS**). Further study showed that these areas were enriched with repressive histone modifications, H3K9me2 and H3K27me3 which had tissue-specific distributions. Additionally, they consisted of few, minimally expressed genes, which were also marked by these repressive histone modifications [11-13].

Genome-wide ChIP analyses have also described other lamina associated domains with significant overlap with the LADS domains. These regions referred as **L**arge **O**rganized **C**hromatin domains of H3**K**9me2 or **LOCKS,** which are also hallmarked with increased

H3**K**9me2 marks, gene-poor and increased in size and abundance from ESCs to differentiated cells (from 4% genome coverage in mouse ESCs to at most 46%, in liver cells) [14]. Wen, Wu et al. described these LOCKs as large regions with K9 modifications up to 4.9 Mb that are conserved between human and mouse. Like LADS, LOCKS also show tissue-specific distributions and inversely correlate with gene expression. Specific knockouts of the H3K9 methyltransferase, G9a, abolished LOCK formation in mouse ESCs and caused gene derepression without any peripheral localization alteration [14, 15]. Although LADS and LOCKS are similar in their effects on gene expression and localization, the relationship between them and their function at the nuclear periphery is presently unclear.

2.2. Bivalency

Since the discovery of generating induced pluripotent stem (**iPS**) cells, the ability to reprogram a differentiated cell back towards a more embryonic-like state, it has been shown by a variety of laboratories that there is an extensive amount of epigenetic variability between different iPS lines and even among different clones. As a result, much research has been involved in understanding the global demethylation and methylation patterns in ESC to identify particular epigenetic marks and their effected genes to study their role in pluripotency and reprogramming. Methylation of DNA cytosine residues (**mCG**), particularly in CpG islands of promoters, is a well-established mechanism that represses gene transcription in adult cells [16]. Several studies have specifically shown in ESCs, that loss of DNA methyltransferases also compromises their ability to differentiate into mature cells without affecting their self-renewal [17].

In embryonic stem cells, lineage-specific gene expression program regulators are repressed, but poised for a rapid response to differentiate [18]. These areas of chromatin, have so called bivalent domains, consist of opposing chromatin marks; i.e. H3K4me for activation and H3K27me for silencing. In ESCs, these domains are believed to be responsible for preventing the transcription responsible for their early differentiation to a specific lineage while priming the area for activation when the appropriate cues are expressed. Consistent with this belief is the findings that the bulk of the protein-encoding genes of human ESCs, including transcriptionally inactive genes have H3K4me, H3K9ac and H3K14ac rich promoter regions in areas of the nucleosome adjacent and downstream of transcription start sites [19, 20]. Moreover, in ESCs, genes with bivalent gene promoters tend to have unmethylated CpG islands [21]. The initial step of active DNA demethylation in mammals occurs by the conversion of 5-methylcytosine of DNA (5mC) to 5-hydroxymethylcytosine (5hmC). A prime example of this in pluripotent stem cells has been shown in regulating the expression of the stem cell transcription factor, Nanog. Here, the demethylated state is critical for the upregulation of Nanog which is an essential regulator of ESC pluripotency and self-renewal, while its downregulation attributed to methylation of its promoter is required for ICM specification [22]. Recent studies demonstrate that demethylation of Nanog is in part contributed to the expression of the Tet methylcytosine dioxygenase 1 (TET1) enzyme which is a TET family member of enzymes that catalyze the conversion of 5mC to 5hmC. This enzyme has been found to demethylate Nanog promoter sites in mouse ES cells [23, 24]. Both TET1 and TET2 expression have also been shown to be regulated by Oct4 expression in mouse ES cells, downregulated following differentiation

alongside other stem cell markers, and is induced concomitantly with 5-hmC during fibroblast reprogramming into iPS cells [25].

In addition to promoter regulation, methylC-Seq genome-wide analysis has also discovered novel types of DNA methylation regulation at non-CG sites (CHG and CHH sites where H = A, C, or T residues). These analyses showed that non-CG methylation accounted for 25% of the total ESC methylome and that these sites were more commonly found within gene bodies than within promoter sites [26]. Furthermore, the methylation of these sites was lost when differentiation was induced in ESCs, and restored during the generation of in induced pluripotent stem cells. This included many differentially methylated regions associated with genes involved in pluripotency and differentiation.

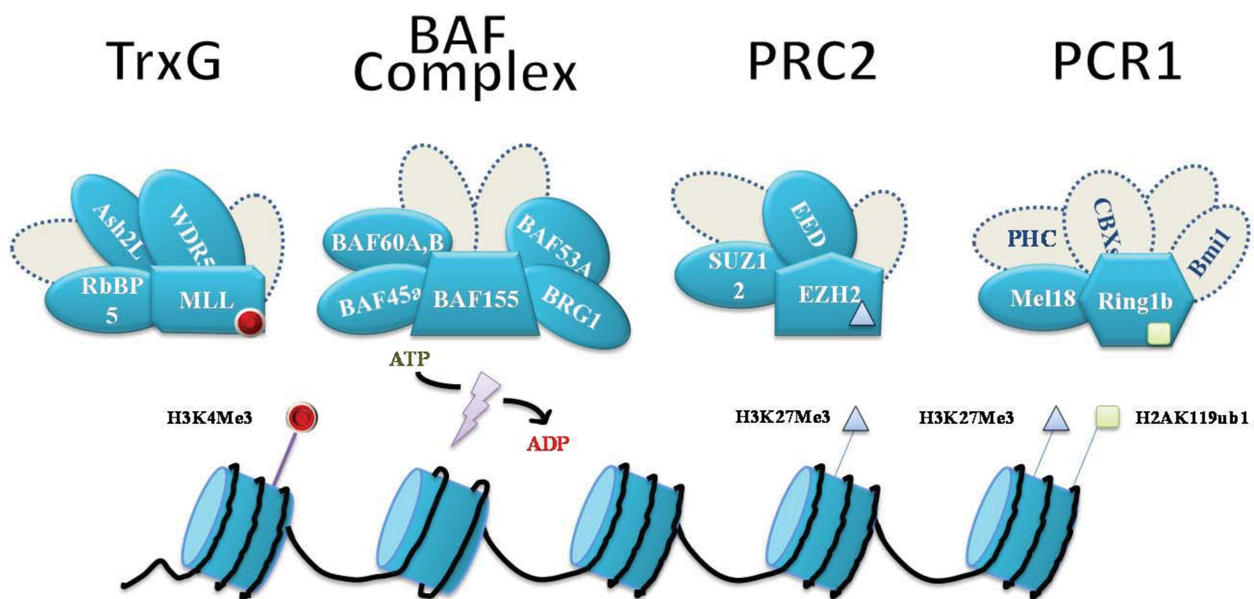
2.3. Polycomb and trithorax group proteins in pluripotent stem cells

Recent studies have established that developmental gene priming and bivalency are crucial for pluripotency whereby the chromatin of pluripotent stem cells are transcriptionally permissive, with normally silent DNA repeat regions, transcriptionally related histone modifications such as H3K9ac, H3K4me3, H3K36me3 and low stochastic transcription of lineage-restricted genes [8, 9]. The poised state is believed to inhibit the activity of RNA Polymerase II (**RNAP II**) and thereby deregulate elongation. In the poised state, RNAP II demonstrates high Ser5 phosphorylation and low levels of Ser2 phosphorylation which is in part controlled by a number of complexes involved in this process. For this, several groups or families of complexes involved in epigenetic regulation have been studied in pluripotent stem cells. These proteins include those which regulate histone modifications, DNA methylation and ATP-dependent chromatin remodelling and include the polycomb group (**PcG**) proteins, trithorax group (**TrxG**) proteins, and ATP-dependent enzymes of the BAF complex. In general, PcG proteins are usually associated with epigenetic gene silencing, while their antagonists TrxG and BAF complexes proteins are involved in epigenetic maintenance or activation of differentiation. The importance of these proteins in developmental regulation of gene expression is well-established [27], while, their functions in adult and pluripotent stem cells is only recently been understood [28]. This includes studies which show that PcG, TrxG, and other chromatin remodeling factors including ATP-dependent enzymes are interconnected in their roles to regulate pluripotency (see Figure 1) [29-33].

PcG, TrxG, and BAF complex associated genes are conserved from fly to man and are important in the regulation of organogenesis and development. PcG proteins were initially discovered as repressors of the Hox or homoeotic genes in *Drosophila,* while TrxG and BAP (BAF *Drosophilia* homolog) proteins maintained Hox gene expression in the appropriate spatial domains. Hox genes encode a family of evolutionarily conserved regulators, which are involved in establishing body segmentation patterns during the development of the fly. In mammalians, these proteins also regulate genes involved in development and differentiation.

PcG proteins produce two distinct protein complexes that act sequentially to regulate gene expression – the “Bmi-1 complex” also known as Polycomb Repressive Complexes (**PRC1**) and the “Eed complex” also known as **PRC2**. The PRC1 or Bmi-1 complex includes Bmi-1, Ring1A/B, Mph1/Rae28, Mel-18, M33, and Scmh1. The PRC2 or Eed complex includes Ezh1, Ezh2,

PHF1, MTF2, Eed, YY1, RBBP4, RBBP7, PCL1, PCL2, PCL3, JARID2, AEBP2, and PHF19 and Suz12 [34, 35]. Initial steps in stem cell reprogramming involves the recruitment of histone deacetylase by activity of the PRC2 complex, which causes local deacetylation of chromatin and subsequent methylation of K27 of histone H3. This H3K27 methylation then recruits the Bmi-1 complex to the site, which leads to the monoubiquitination of Lys119 histone H2A, and in turn suppresses gene expression [36]. The coordinative action of these two complexes plays an important role in the regulation and maintenance of gene expression during development and contributes to the epigenetic memory of stem cells [37, 38].



BAF complex > Nature Neuroscience 13, 1330–1337 (2010) doi:10.1038/nn.2671 Published online 26 October 2010
TrxG & PRCs : *Cell Stem Cell.* 2012 Jul 6;11(1):16-21. doi: 10.1016/j.stem.2012.06.005.

Figure 1. Chromatin remodeling factors of the TrxG, PRC1/2 and BAF complexes work together to regulate stem cell status. In pluripotent stem cells, genes necessary for lineage-specific regulation consist of 'bivalent' chromatin domains that contain repressed H3K27me3 marks, as well as active H3K4me3 marks. These genes are then 'primed' for rapid induction of expression upon receiving differentiation cues. Proteins of the TrxG family tri-methylate H3K4 leading to active chromatin marks. PRC2 activity leads to repressive tri-methylation of H3K27 and subsequent recruitment of PRC1 to the nucleosome region. Upon recruitment, PRC1 transfers a mono-ubiquitin residue to histone 2A (H2AK119). Together, the binding of PRC1 and the ubiquitylation of H2AK119ub silences gene expression. BAF complexes directly unwind nucleosomal DNA by using ATP and helicase-like subunits. Together, these complexes coexist and/or work hierarchically to regulate pluripotency and bivalency in stem cells.

Polycomb repressive complexes have been shown associated with many developmental regulator regions in ESCs, and many of the PcG repressed targets of ESCs are also 'bivalent' [30, 39]. For instance, PRC2 target genes have been shown to be preferentially turned on during ESC differentiation and that the pluripotent stem cell regulating genes Oct4, Sox2, and Nanog co-occupy a significant subset of these genes. Therefore, it has been suggested that the PRC2

complex represses a distinct group of developmental genes that have to be repressed to maintain pluripotency. This would promote a poised or primed state which could be readily activated during early differentiation [40]. For example, the histone methyltransferase Ezh2 is known to catalyze H3K27me3. In fact, bivalency domains at PRC2 regulated promoters are roughly five times more likely to become DNA methylated during differentiation than those with non-PRC2 regulated promoters [21] suggesting that the PRC2 complex plays a pivotal role in the switch for early lineage commitment [41]. Jarid2, a member of the Jumonji family of histone demethylases, has also been shown to play an important role in properly recruiting PRC1 and PRC2 and initiating the RNA Polymerase II activating form (Ser5P-RNAPII) [42] to bivalent loci to promote differentiation [43-45]. While Jarid2 is enzymatically inactive in ESCs, recent evidence has shown that Jarid2 is regulated by pluripotency factors in ESCs [43]. In null ESCs lacking Jarid2 expression were able to self-renew but unable to differentiate despite expressing appropriate PRC2 target genes demonstrating that transcriptional priming of bivalent genes in ESCs was dependent on Jarid2 expression.

In addition to the bivalent marks associated with PRC2 associated H3 modifications in pluripotent stem cells, bivalent genes in stem cells also exhibit repressive marks of H2AK119Ub1 at their promoter and throughout the coding region. For this reason, members of the PRC1 complex Ring1A and Ring1B proteins which catalyze H2AK119ub1 have also reveal a role in regulating pluripotency [30, 46]. For instance, in ESCs, double mutants of Ring1A/B demonstrate reduced levels of H2AK119Ub1, repression of known stemness genes (including Oct4 targets), increased expression of developmental regulator targets, and spontaneous differentiation. Furthermore, upon differentiation, Ring1A/B lost binding to their target loci suggesting that a Ring 1/B mediated complex functions downstream of the stem cell core transcriptional machinery to maintain the ESC state [47].

In addition to PcG complexes, core members of the TrxG and BAF chromatin remodeling complexes have also been shown to contribute to the bivalent mark in stem cells by acting in concert to establish and preserve H3K4me3 [48, 49]. Another core member, WD repeat domain 5 (**Wdr5**) of the TrxG complex, has also been associated with the undifferentiated state and shown to regulate self-renewal in mouse ESCs [50]. This study went further to show that Wdr5 expression can promote efficient derivation of pluripotent iPS cells. Along with PcG and TrxG complexes, other chromatin remodeling complexes have been shown to have crucial roles in maintaining pluripotency. For instance, it has been shown that ESCs contain a unique BAF complex, which distinguishes them from differentiated cell types. This ESC complex consists of BAF60a, BAF155 and BRG subunits in the absence of BAF60c, BAF170 or BRM [48, 51]. Components of this ESC BAF complex also appear in RNAi screens for pluripotent genes [52, 53]. In mouse ESCs, it was also shown that BRG removal causes decreased self-renewal and aberrant differentiation, whereas BRG withdrawal from differentiated cells had very little effect [51]. Additionally, the pluripotency regulator genes Oct4, Sox2 and Nanog, have been shown to be targeted by components of the ESC-specific BAF complex [48, 51, 54-56] as well as facilitate IPS cell reprogramming [33].

3. Epigenetic control in primordial germ cells and pluripotent embryonic germ cells

Primordial germ cells (**PGCs**) are the progenitors of sperm and egg. In humans, these cells first appear around the fifth week after conception and in mouse, during embryonic day (**E**) 5. At this time, PGCs emerge from differentiated epiblast cells which have already begun to undergo major epigenetic changes including DNA methylation and X inactivation [57]. Thus, epigenetic regulation or reprogramming must occur in PGCs in order to achieve an undifferentiated totipotent-like state [57, 58]. X activation and demethylation in PGCs is similar to that seen in the process of generating ESCs and reprogramming somatic cells into pluripotent iPS cells [59]. Together, these epigenetic changes in germ cell development is to prevent the transmission of aberrant epigenetic modifications to the next generation and to promote epigenetic equivalency in the germ line of male and female embryos, which is necessary for proper imprinting. This is the only time in which homologous chromosomes are epigenetically indistinguishable and in PGCs occurs primarily in the developing embryonic gonad [60, 61].

Key initiators of PGC induction in the epiblast include the Blimp1 (**B-lymphocyte-induced maturation protein 1**), also known as PR domain zinc finger protein 1 (**Prdm1**), Prdm14 and protein arginine methyltransferase 5 (**Prmt5**). These factors have been shown to initiate epigenetic reprogramming and induce repression of the somatic program during germ cell specification [62-64], and in a similar fashion to facilitate somatic cell reprogramming in iPS cells and in epiblast stem cell generation [65, 66]. Their effects occur through both direct and indirect targets. For instance, it is known that BLIMP1 associates with the arginine methyltransferase PRMT5 to reduce expression of Hox-family genes and other somatic genes in PGCs via H2A/H4 R3 methylation [67].

In addition to pluripotent associated genes, early germ-cell development and imprinted genes also undergo demethylation during this time. These include well-established postmigratory germ cell genes Mvh (also known as Ddx4), Sycp3 (synaptonemal complex protein 3) and Dazl (deleted in azoospermia-like). These genes are demethylated in germ cells and repressed in somatic cells. This demethylation occurs during the migration of PGCs into the gonad at CpG islands of their promoters as well as at differentially methylated regions (**DMRs**) of imprinted genes [68, 69]. Whether DNA methylation in PGCs is erased by an active or a passive mechanism is currently unclear. However, two DNA deaminases, activation-induced cytidine deaminase (**AID**) and apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1 (**APOBEC1**) may demonstrate a key role in this process. While both enzymes have been shown *in vitro* to deaminate 5-methylcytosine in DNA in mouse PGCs, deficiencies in AID expression has also shown that it is essential for erasure of DNA methylation [70]. Both, Aid and Apobec1 are located in a cluster of genes which comprise Stella, Growth differentiation factor 3 (**GDF3**) and Nanog. Stella, Gdf3 and Nanog are all expressed in pluripotent tissues as well as in germ cells. [71]. While Stella is a known constituent of germ cell development, Gdf3 and Nanog have important roles in conferring stem-cell identity on ES cells. It has also been suggested that *in vivo* targeting in the zygote of AID to the methylated DMR of the imprinted gene H19 results in efficient and substantial demethylation of this region [72]. Together these findings suggest

an important role of AID in facilitating demethylation and reprogramming the pluripotent state. Similar to AID, TET1 and TET2 may also play a facilitative role in PGC demethylation as both TET enzymes are expressed in mouse PGCs during imprint erasure, concurrent with 5hmC enrichment [73].

Another important epigenetic process required for germ cell development and cellular reprogramming to the pluripotent state involves the X chromosome. In female adult cells, one of the two X chromosomes is inactivated to compensate for the differences in gene expression between sexes. For this purpose, X chromosome inactivation is initiated in early embryos by noncoding X-inactive specific transcript (Xist) RNA followed by chromatin modifications on the inactive X chromosome which leads to stable gene repression in somatic cells. Likewise, reactivation of the X chromosome is required for the totipotency of the female blastocyst and germ cell development. Reactivation of the X chromosome also occurs to establish pluripotency in iPS cells. During development, epigenetic reprogramming or re-activation of the inactive X-chromosome commences in PGCs during their migration through the hindgut along their route to the developing gonads where imprint erasure is completed [74]. In mouse PGCs, decreased Xist expression, and the displacement of PcG repressor proteins EED and SUZ12, results in the loss of the inactive X associated histone modification, H3K27me3 [74]. In humans, PcG proteins YY1, EZH2, and EED have also been found in the ovarian follicles, oocytes and preimplantation embryos. YY1 and EZH2 transcripts were additionally detected in human metaphase II oocytes suggesting they may be play a similar role in human germ cell reprogramming [75].

In vitro, PGCs cultured under specific conditions can also demonstrate epigenetic reprogramming with pluripotent cell-like characteristics. In these cases, PGCs form pluripotent stem cell colonies called embryonic germ cells (EGCs) which have notable similarities in their epigenomes [58, 76-82], and like ESCs, EGCs have been shown to induce epigenetic reprogramming of somatic nucleus in hybrid cells [60].

4. MicroRNAs and stem cells

MicroRNAs (**miRNAs**) are a family of non-protein coding RNAs with transcripts of ~20–25 nucleotides that play essential roles in regulating gene expression (see [83-85]. A subset of miRNAs have been shown to be preferentially expressed in undifferentiated stem cells and for some, have been shown to play essential roles in pluripotency, proliferation, and modulation of expression patterns that are related to differentiation [86-89]. The promoter regions of these miRNAs are often occupied by the pluripotency transcription factors, including Oct4, Sox2, and Nanog [90]. In addition, during ESC differentiation, proteins modulated by miRNAs [91] have also been shown to be modulated by PcG proteins [92]. For instance, Marson *et al.* showed that approximately one quarter of the Oct4/Sox2/Nanog/Tcf3-occupied miRNAs belonged to a set of repressed miRNA genes bound by Suz12 in murine ES cells [90]. Here, the PRC2 complex protein SUZ12 was bound to a subset of inactive miRNAs controlling differentiation in mouse ESCs [90]. In this study, SUZ12 bound to the promoters of several miRNAs

associated with repressing differentiation in ESCs. In a similar fashion, another PRC2 associated factor, the transcription factor YY1 has also been shown to directly regulate miR-29 transcription through the recruitment of HDAC1 and EZH2 to the regulatory regions of the miR-29 promoter [93].

Studies in mice have shown that induction of neural differentiation in mouse ESCs with retinoic acid results in increased miR-134, miR-296, and miR-470 which in turn interact with the coding sequences of the pluripotency transcription factors Oct4, Sox2, and Nanog. These results suggest that through interaction of the miRNAs these pluripotent stem cells genes are downregulated thereby permitting differentiation to proceed [91]. Additionally, the ESC-specific miR-290 cluster has also been shown to regulate Oct4 methylation in differentiating ESCs [94]. Other studies have shown that mouse ESCs deficient in proteins of the miRNA processing apparatus such as Dicer, Drosha, DGCR8, and Ddx5 exhibit differentiation and developmental defects [95-97].

Interestingly, PcG proteins have been shown to be both regulators of miRNA expression as well as their targets. For instance, miRNA-101 has been shown to directly regulate the expression of the PRC protein EZH2 in highly aggressive cancers [98, 99].

5. Epigenetic regulation in progenitor and adult stem cells

Progenitor cells and adult stem cells are thought to be predecessors of pluripotent or multipotent stem cells that are generated during early differentiation. During their transition in development, bivalently marked stem cell genes can become either active, or inactive, or remain bivalent, dependent in part, on the activity of key enzymes which drive these chromatin modifications such as lysine demethylases (**KDMs**), histone deubiquitylases (**DUBs**), and DNA methyltransferases (**DNMTs**). Bivalent chromatin, although present in progenitor cells, is less frequent than in pluripotent stem cells. This has been shown in mesenchymal stem cells, hematopoietic stem cells and neural progenitors, in which cases the bivalency continues to resolve upon further differentiation [100]. During this process in pluripotent stem cells, active genes exhibit diminishing suppressive chromatin marks, an increase in H3K4me3, gain of H3K36me3 within coding sequences of DNA, and contain RNAP II that carries high Ser5 phosphorylation and low levels of Ser2 phosphorylation near promoter and within coding regions. Moreover, inactive genes show loss of active chromatin marks while retaining repressive ones, and in some cases gaining CpG methylation (mCpG).

Specific differences occur in the chromatin states between pluripotent stem cells, progenitor cells and more differentiated cell types which include active, repressed and poised states of chromatin. Several lines of evidence suggest that priming in the poised state enables genes to respond rapidly when differentiation cues are presented [30]. For example, during neural induction, several hundred genes including those required to maintain stem cell-ness become de novo mCG and therefore transcriptionally silenced. Furthermore, the observation was made that neural precursors that are derived from ESCs acquired more mCG than terminal

neurons, suggesting that the transition from pluripotent to lineage-committed cells is associated with these changes [17, 21, 101, 102].

Polycomb group proteins also appear to play a unique role in defining the progenitor or adult stem cell state. It has been shown that the PRC1 complex protein Bmi-1 activates multiple pathways that are important for regulating the stem cell-like state. For example, it has been shown that Bmi-1 is potentially upregulated via the pluripotent stem cell marker SALL4 signaling and has been shown to regulate stem cell self-renewal by repressing Hox genes, as well as INK4a locus genes, p16INK4a and p19ARF. BMI1 has also been shown to facilitate stem cell-like features in adult stem cells such as increased telomerase activity, transcriptional factor GATA3, and NF-kB pathways. These pathways are associated with the prevention of senescence, differentiation and apoptosis, while promoting immortalization and proliferation (for review see [103]).

6. Epigenetic dysregulation in cancer stem cells

Cancer stem/initiating cells (CSC) have been defined as a subset of cancer cells that have clonal ability or self-renewal and are resilient against cancer therapies [104, 105]. As such CSCs are implicated in cancer initiation, metastasis, and recurrence of some cancers [106]. Although the most well established pluripotent stem cell genes OCT4, NANOG, cMYC and SOX2 are implicated in many poorly differentiated or metastatic cancers [107-109], they are not expressed in all and they are not all elevated concordantly. In addition, targets of NANOG, OCT4, SOX2, and c-MYC are often overexpressed in tumors that are poorly differentiated, more so than in those that are well differentiated [110]. These genes also play a significant role in the induction of pluripotency into iPS cells from differentiated cell types and are thus involved in regulating epigenetic reprogramming [111-113]. More specifically, it is found that c-MYC, which is also an oncogene is sufficient for the reactivation of ESC-like transcriptional program in both, normal and cancer cells [114]. Additionally, studies have shown that one of the inherent issues with generating iPS cells is their propensity to become cancer stem cell-like [115, 116]. Taken together, these results indicate that aberrant activation of an ESC or iPS-like transcriptional program might cause induction of pathological self-renewal in adult differentiated cells, characteristic of cancer stem cells.

Aberrant function of PcG proteins has also been established in the malignancy of various cancers [117]. This is not surprising as it is well known that polycomb complexes contribute to the epigenetic regulation of key networks associated with self-renewal [118], differentiation, and proliferation [92, 119-123]. These roles for polycombs have been demonstrated in cancer cells and normal stem cells [124] and more recently studied for their targeted function in CSCs [125]. For instance, there is much evidence that overexpression of the EZH2 polycomb gene occurs in multiple human malignancies (see [117, 126]). One study showed that this may in part be atributed to a genomic loss of miR-101 which has been shown to lead to increased EZH2 levels [99, 127]. Although how EZH2 contributes to carcinogenesis remains poorly defined, recent evidence suggests that overexpression of EZH2 can contribute to improper silencing of

tumor suppressor genes [121]. In this case, EZH2 was shown to target a pro-differentiation tumor suppressor gene, retinoic acid receptor β2 (**RARβ2**) [120], which is reduced or lost in many human malignancies.

7. Conclusion

The pluripotent stem cells have a chromatin that is hyperdynamic, with a preponderance of modified histones and chromatin remodelers that ensures low-level transcription and tight regulation. Losing pluripotency is accompanied with a more compact, repressive, chromatin structure, which leads to cellular differentiation. Chromatin architecture is regulated at multiple levels in conjunction with known pluripotent genes to constitute an interwoven pluripotency network. Although there are many gaps in our knowledge of how epigenetic modifications regulate the pluripotent state, it is known that PcG repressor proteins prevent the precocious expression of lineage-restricted gene expression in pluripotent stem cells and germ cells by contributing to a unique 'primed' bivalent state of the chromatin. Future studies will provide mechanistic insights into the signaling cues required to maintain this state and inhibit differentiation while iPS cells and adult stem cells provide a renewed opportunity to study the role of chromatin architecture for controlling the pluripotent state. This will include understanding the mechanisms that interplay between pluripotent transcription factors, epigenetic regulators, and miRNAs to balance self-renewal and differentiation, properties which regulate reprogramming and carcinogenesis.

Nomenclature

cdk, cyclin-dependent kinase; H2A-K119-Ub, ubiquitinylated histone H2A lysine 119; H3K27me3, tri-methylated histone H3 lysine K27; PcG, Polycomb group genes; ESC, embryonic stem cells; EGC, embryonic germ cells; PGC, primordial germ cells; iPS, induced pluripotent stem cells; CSC, cancer stem cells; RARβ2, retinoic acid receptor β2; Hh, Hedghog; KDMs, lysine demethylases; DUBs, histone deubiquitylases; DNMT, DNA methyltransferases; YY1, Ying Yang 1; EZH2 Enhancer of Zeste-2; EED, embryonic ectoderm development; GDF3, growth differentiation factor 3; DMR, differentially methylated regions; DAZL. deleted in azoospermia-like; Mvh, deadhead box 4; Sycp3, synaptonemal complex protein 3; E, embryonic day; RNAP II, RNA Polymerase II; trxG, trithorax group proteins; AID, activation-induced cytidine deaminase; **APOBEC1,** apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1

Acknowledgements

This work was supported by NIH grants R01 AR053851 and R01 CA131074 to R Eckert and R21HD057487 awarded to C Kerr as well as by the State of Maryland Stem Cell Research Fund 2010-MSCRFI-0110-00 to R Eckert and 2007-MSCRFII-0159-00 awarded to C Kerr.

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