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Diverse heterochromatin states restricting cell identity and reprogramming

Ryan L. McCarthy,

Jingchao Zhang,

Kenneth S. Zaret

Institute for Regenerative Medicine, Penn Epigenetics Institute, Dept. Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

Abstract

Heterochromatin is defined as chromosomal domains harboring repressive H3K9me2/3 or H3K27me3 histone modifications and relevant factors that physically compact the chromatin. Heterochromatin can restrict where transcription factors bind, providing a barrier to gene activation and cell identity changes. While heterochromatin thus helps maintain cell differentiation, it presents a barrier to overcome during efforts to reprogram cells for biomedical purposes. Recent findings reveal complexity in the composition and regulation of heterochromatin and that transiently disrupting the heterochromatin machinery can enhance reprogramming. Here, we discuss how heterochromatin is established and maintained in development and how our growing understanding of the mechanisms regulating H3K9me3-heterochromatin can be leveraged to improve our ability to direct changes in cell identity.

Keywords

heterochromatin; H3K9me3; H3K27me3; reprogramming; pioneer factors

Heterochromatin: Restricting Access to the Genome

Despite all cells containing the same genetic information, each cell type in multicellular organisms expresses a subset of genes corresponding to its distinct cellular function. The expression of cell type specific genes relies upon transcription factors acting in the context of chromatin. During development, the progressive expression of sets of transcription factors drives changes in cell identity and lineage commitment. Reprogramming involves the activation of a new cell identity, typically by the ectopic expression of a cocktail of transcription factors that activate alternative lineage genes. The ability to reprogram cells was originally discovered with the observation that nuclear transfer can change cell

Declaration of interests

No competing interests are declared.

identity [1]. Reprogramming through the direct expression of transcription factors was first demonstrated by the ability of MyoD to convert fibroblasts to myoblasts [2] and later shown by reprogramming of B cells into macrophages through expression C/EBPα and C/EBPβ [3]. Finally, fibroblasts were converted to pluripotent stem cells following expression of the Oct4, Sox2, Klf4 and c-Myc (OSKM) transcription factors [4]. Reprogramming from one somatic cell lineage to another somatic cell lineage, also referred to as trans-differentiation, has been used to generate many cell types including hepatocytes [5], cardiomyocytes [6] and neurons [7].

Transcription factors can be restricted from binding to heterochromatic regions of the genome that are compact, inaccessible, and hence transcriptionally silent. By contrast, euchromatin is more open, accessible, and generally transcriptionally active. Transcription factors vary in their abilities to bind to free DNA, euchromatin, and silent, unmarked chromatin regions, but are largely blocked from activating target genes in heterochromatin regions during reprogramming [8,9]. Thus, learning how to overcome heterochromatin repression to enable transcription factor binding helps improve our ability to reprogram cells for basic science and therapeutic applications [10–12].

Here we review studies revealing an emerging view that heterochromatin is complex in composition. After reviewing such complexity, we will focus on the H3K9me3-heterochromatin subtype in mammalian cells, including how it is established and rearranged during early development, how it resists activation during reprogramming, and how it can be disrupted to enhance reprogramming (Figure 1). It appears that H3K9me3-heterochromatin achieves gene silencing through diverse mechanisms, resulting in structures and biochemical parameters that may interact differently with specific classes or families of transcription factors. Unraveling such specificity is a major goal for the future.

Diverse Types of Heterochromatin

Functionally, heterochromatin silences alternative lineage genes during development [13–16], maintains repression of repeat elements, and promotes genome stability by suppressing recombination among different repeats across the genome [17]. The repressive function of heterochromatin is driven by its structure, biochemical modifications, and chromatin associated proteins and RNAs.

Our understanding of the structure of heterochromatin has undergone a dramatic shift, thanks to new insights provided by novel imaging, genomics, and biochemical advances. Compared to the uniform nucleosome compaction observed *in vitro*, recent experiments *in vivo* revealed a more complex picture of heterochromatin structures, with heterochromatin assuming multiple nucleosome configurations [18,19] and forming various higher-order structures [20]. Integrating how chromatin structural configurations correspond to specific histone modifications, protein and genomic compositions and their impact on transcription factor binding will provide key insights into heterochromatin regulation and function in development and reprogramming.

Heterochromatin is often characterized by the associated biochemical modifications that decorate the DNA and histones. The first heterochromatic mark discovered was DNA methylation, which is generally associated with transcriptional repression when occurring at CpG islands of gene promoters, but its function depends upon genomic context [21]. Covalent modification of the histone tails, including di- and tri-methylation of histone 3 lysine 9 (H3K9me2/3) [22], and tri-methylation of histone 3 lysine 27 (H3K27me3), are the most extensively studied histone modifications associated with heterochromatin. H3K27me3, catalyzed by Polycomb Repressive complex 2, has been associated with heterochromatin at developmental genes, including Hox clusters, which are dynamically regulated during development [23,24]. H3K9me2, catalyzed by the histone methyltransferases (HMTs) G9a/GLP, and H3K9me3, catalyzed by the HMTs SETDB1 and SUV39H1/H2 respectively [22] have long been known to repress repetitive elements. H3K9me2 and H3K9me3 are differentially distributed in the nucleus, with H3K9me2 signals mainly detected at the nuclear periphery and interacting with nuclear lamins through adaptor proteins [25,26], and H3K9me3 detected at both nuclear periphery and other more centrally located heterochromatin compartments, such as peri-nucleolar and pericentric heterochromatin [27]. Upon loss of H3K9me3 in *C. elegans*, H3K9me2 can maintain repression at some previously H3K9me3 repressed genes and repeats, but not all, indicating overlapping but not redundant repressive function [15]. Growing evidence has shown that H3K9me2/3 is dynamically regulated at genes and enhancers during development to enable lineage specifications and restrict alternative lineages [13,22,28,29]; H3K9me3 will be the major focus of this review. Additional repressive marks including H4K20me3 [30,31], H3K64me3, H2AK119ub1 [24,32], and histone variants [33], together contribute to the complex organization and regulation of heterochromatin.

H3K9me3-heterochromatin can be further decorated by associated proteins and RNAs, to enforce repression. Linker histone H1 associates with the “linker” DNA region between nucleosomes throughout most of the chromatin, i.e., both euchromatic and heterochromatin, but a higher density of H1 in heterochromatin domains contributes to chromatin compaction [34,35]. So-called histone modification-reader proteins include the heterochromatin binding proteins HP1α, HP1β, and HP1γ, which bind methylated lysines through their chromodomain and recruit SUV39H1/H2 and SETDB1 to spread H3K9me3 marks to neighboring nucleosomes, compacting the chromatin, and reinforce repression through the cell cycle [36]. Chromatin associated non-coding RNAs also play important roles in establishing and maintaining heterochromatin, such as the Xist RNA in X chromosome inactivation [37], satellite RNAs in the recruitment of SUV39H1 and SUV39H2 [38,39], pseudogene lncRNAs in the recruitment of SUV39H1 [40], and endogenous siRNAs which recruit HMTs through nuclear Argonaute [41]. Specific protein compositions of different heterochromatin compartments [8,37,42] may explain how heterochromatin can be uniquely deposited and rearranged in development and reprogramming.

Heterochromatin remodeling enables zygotic genome activation and totipotency

Mammalian embryos undergo extensive epigenetic reprogramming during pre-implantation development, erasing epigenetic information from the past generation and establishing new epigenetic programs to enable developmental progression [43]. Therefore, early development offers an important model to investigate molecular mechanisms of heterochromatin initiation, establishment, and maintenance, and its impact on cell potential (Figure 2).

In the zygote, the paternal genome in sperm is largely packaged with protamines, while the remaining canonical histones are largely devoid of H3K9me3 [28,44]. The zygotic maternal genome possesses canonical H3K9me3-, H3K40me3-, and H3K64me3-marked heterochromatin at centromeric, pericentromeric, and telomeric regions [44,45]. De novo H3K9me3 on paternal genomes by SUV39H2 starts as early as the late zygote stage [46], although the association of the SUV39H2 RNA-binding domain with the pericentromeric RNA transcribed from the paternal genome limits its methyltransferase activities [46,47]. SUV39H1 lacks RNA binding domains [39] and overexpression of SUV39H1 induces precocious H3K9me3 heterochromatin in zygotes, causing a developmental arrest at the 2-cell stage and reducing nuclear transfer efficiency by the oocyte [46,48]. Similarly, depleting KDM4a in oocyte, the major H3K9me3 demethylase expressed in mouse and human oocytes, leads to invasion of H3K9me3 domains into euchromatin and disrupts zygotic gene activation [49]. We can conclude that precisely coordinated heterochromatin resetting is crucial for establishing a permissive chromatin environment for zygotic genome activation and establishing totipotency (Figure 2).

Heterochromatin re-establishment in early embryo drives the transition from totipotency to pluripotency

Heterochromatin maintains genome integrity by preventing the recombination between repeat sequences and silencing transcription from repetitive elements to prevent the formation of RNA:DNA hybrids (reviewed in [17]). However, the newly established heterochromatin domains before the 8-cell stage lack HP1α [30,50] and most of the linker histone H1 variants [51], which normally are molecular hallmarks of compact heterochromatin domains [34], consistent with the notion that heterochromatin domains prior to the 8 cell stage harbor a noncanonical, non-repressive structure [52]. Consequently, the resetting of H3K9me3, along with erasure of other heterochromatin marks, H3K64me3 and H4K20me3, and DNA methylation (reviewed in [53]) from the 2-cell to blastocyst stage leads to transient activation of satellite repeats and many retrotransposons and during pre-implantation development [54].

Interestingly, a pulse of major satellite RNA transcribed from the paternal genome during the zygote stage recruits SUV39H2 to pericentromeric regions [39] and the transcription from both strands may lead to dsRNA formation, reminiscent of RNAi mechanisms in *S. pombe* and *C.*elegans (reviewed in [22]). Retrotransposons constitute a large proportion

of the mammalian genome, and mounting evidence suggests that RNAs transcribed from diverse classes of retrotransposons can direct different heterochromatin machineries to silence the repetitive DNA and target genes [55–58] (Figure 2B). The retrotransposons and can be broadly divided into non-LTR elements, including LINE and SINE elements, and LTR elements, including ERV1, ERV2, ERV3 and MaLR (reviewed in [59]). LINE elements constitutes 10%−30% of eutherian genomes [60] and its transcripts, abundant in 2-cell embryos, recruit Nucleolin and KAP1 (TRIM28) to repress Dux, master regulator of 2-cell totipotency genes, and therefore drive the exit from totipotency [56].

In addition, the HUSH complex recognizes L1 (LINE) RNAs and recruits SETDB1 to silence L1 retrotransposons, although direct evidence for a function of the HUSH complex in early development is still lacking. LTR elements represent around 25% of retrotransposons in the mammalian genome, and their transcripts are detected from zygote to morula stages, some of which show remarkable stage-specificities (reviewed in [60]). ERV2 families, including IAP and ERVK are among the most abundant ERV elements in the mouse genome [59,61]. RNA m6A modifications by Mttl3/4 on IAPs RNAs can mark the RNAs for degradation [58] and recruit YTHDC1, which further recruits Setdb1 to initiate heterochromatin formation at IAP elements [55]. It is currently unknown if the RNA-directed mechanism also plays a role in silencing other LTR families.

It is possible that RNA transcribed at heterochromatin domains may directly recruit HP1 proteins through interactions with the HP1 hinge domains [62]. Taken together, the extant studies indicate that RNA is at the core of heterochromatin initiation and maintenance and provides targeting specificity for heterochromatin machineries to silence diverse repeat families. Recently, of 172 proteins found to be associated with H3K9me3-heterochromatin in human fibroblast cells, many are strongly enriched for RGG RNA-binding motif [8], hinting that the RNA binding is a common mechanism for heterochromatin formation and maintenance.

In addition to the RNA-directed heterochromatin initiation mechanisms mentioned above, many transcription factors directly interact with SUV39H1/H2, SETDB1, and HP1 to recruit heterochromatin machinery to repress diverse retrotransposon families and lineage specific genes (Figure 2D) [63–65]. KRAB-ZFP proteins represent a repertoire of constantly evolving transcription factors that recruit SETDB1 through the bridging factor KAP1 to silence invading retrotransposons (reviewed in [66]). Some zinc-finger proteins, including ZFP809 [67], KLF4, KLF17 [68], and ZFP93 [69], are highly expressed in early embryos and bind to specific families of retrotransposons, indicating that ZFPs can recruit H3K9me3 machineries to establish H3K9me3 heterochromatin at specific retrotransposons in early development. Interestingly, the maturation of heterochromatin domains requires additional heterochromatin associated proteins, including CAF-1, linker histone H1 and SUMOylation pathway (Figure 2C) [46,56,70]. Depleting SETDB1 and the aforementioned heterochromatin associated proteins in early embryos causes a developmental arrest at the 2-cell stage and de-represses totipotent genes Dux and Zscan4 in pluripotent ES cells, causing reversion to 2 cell-like totipotent state. Therefore, the re-establishment of H3K9me3 heterochromatin directed by RNA and TFs plays important roles in repressing the 2C totipotency program and driving the transition to pluripotency at the blastocyst stage.

Dynamic heterochromatin changes enable lineage specification in development

In addition to repressing retrotransposons, H3K9me3-marked heterochromatin plays important roles in delineating lineage specification during and after gastrulation (Figure 2D–E) [13]. Mapping H3K9me3 changes at protein-coding genes, from the germ-layer stage to endoderm progenitors, and then to differentiated hepatic and pancreatic cells, reveals that, in addition to the expected acquisition of H3K9me3 at genes that become silent in terminal differentiation, surprisingly many genes are marked by H3K9me3 heterochromatin at the germ layer stage and gradually lose the mark during lineage progression (Figure 2E). Further genetic studies with Suv39h1/Suv39h2 and Setdb1 triple KO or Setdb1 knockdown show that H3K9me3 heterochromatin functions to restrict late developmental genes and repress alternative lineages [13,28] (comprehensively reviewed in [22]). Thus, H3K9me3 dynamics at protein coding genes are critical for embryologic differentiation to progress properly. Interestingly, in addition to the roles of ZFPs in heterochromatin initiation mentioned above, some of the KRAB-ZNF proteins also show lineage specific expression and functions, such as ZNF417/ZNF587 in human neurons [71], ZNF558 in human neural progenitors [72], ZNF589 in human hematopoietic system [73], and ZNF808 in human pancreatic development [74]. Thus, that KRAB-ZNF proteins and the transposable elements that they target can be co-opted by the host genome to expand the lineage and species-specific regulatory network [75,76].

In summary, H3K9me3-heterochromatin dynamics are critical for early development. Interestingly, the drastic heterochromatin remodeling in early development does not necessarily lead to genome instability, and similarly no genome-wide genome instabilities in liver were observed after global loss of H3K9me3 caused by compound SUV39H1/2 and SETDB1 deletions [13], suggesting that the roles of H3K9me3 heterochromatin in safeguarding genome stabilities are cell context dependent. Understanding how different heterochromatin associated proteins direct diverse heterochromatin patterns during development has inspired novel screens to perturb heterochromatin machineries to help reprogram cells [10].

Heterochromatin blocks transcription factor binding and gene activation

To elicit cellular reprogramming, transcription factors are induced to bind and activate genes of a new cell identity. Many reprogramming protocols have been developed to enable conversion to diverse cell identities, including pluripotent stem cells [4], macrophages [3], hepatocytes [5], cardiomyocytes [6] and neurons [7]. However, in most cases, the reprogramming elicited by the ectopic expression of transcription factors is limited and does not reflect the desired, fully differentiated cell state [77]. Indeed, reprogramming transcription factors are often impeded from binding terminal differentiation genes of alternative fates because of repressive chromatin at important differentiation genes, particularly H3K9me3-heterochromatin [8] [9,15,78]. Transcription factors possess different capacities to bind and open closed chromatin.

Pioneer transcription factors have DNA binding domains that can bind a partial motif displayed on the surface of a nucleosome [79], leading to chromatin opening and enabling additional factors to bind [80]. Hence pioneer factors can scan closed chromatin regions, in contrast to transcription factors that primarily target open chromatin regions [81,82]. Analysis of heterochromatin compartments and diverse transcription factors by single molecule tracking demonstrated that the pioneer transcription factors' nonspecific DNA and nucleosome binding ability enabled access to the most restricted heterochromatin [83]. Loss of OCT4 nucleosome binding ability, without compromising free DNA binding affinity, was sufficient to exclude OCT4 from binding closed chromatin and abolish its reprogramming capacities [84]. Therefore, pioneer factor binding initiates structural changes between the DNA and histones [85,86] and facilitates binding of other transcription factors and remodelers [87].

Despite their abilities to bind nucleosomes, the pioneer transcriptional factors Sox2 and Oct4 are largely excluded from the H3K9me3 marked heterochromatin during reprogramming [9,88]. For instance, in human pluripotent cells Oct4, Sox2 and Klf4 are bound to pluripotency genes such as Nanog and Prdm14, but these genes are buried in H3K9me3-marked heterochromatin domains in human fibroblasts. The activation of such pluripotent genes in H3K9me3 heterochromatin occurs at the final stage of iPSC reprogramming and is a rate-limiting step. Similarly, during fibroblast to hepatic cell reprogramming by pioneer factor FoxA3 with transcription factors HNF1α and HNF4α, hepatic genes repressed by H3K9me3-marked heterochromatin are more resistant to activation than the genes marked by H3K27me3 or silenced chromatin marked by neither H3K9me3 and H3K27me3 [8]. During pro-opiomelanocortin to melanotropes differentiation, binding of the pioneer factor PAX7 was also blocked from regions with high H3K9me3 [89]. H3K27me3 heterochromatin can also block MyoD in undifferentiated muscle cells [90] and multiple lineage specific transcription factors during early mouse and human embryonic stem cell differentiation [91]. Although heterochromatin has been shown to exclude transcription factor binding in many cell contexts, the ability to bind or being excluded from specific chromatin contexts varies among specific pioneer factors [83,92].

Heterochromatin can be de-repressed to enhance reprogramming gene activation

Loss of all H3K9 methylation through disruption and deletion of all H3K9 lysine methyltransferases leads to global chromatin decompaction, including loss of electron-dense heterochromatin and derepression of protein-coding genes and repeat elements [93]. Transiently depleting diverse non-enzymatic proteins important in maintaining H3K9me3 enhances activation of genes in heterochromatin and improves reprogramming [8–10,12,15,94] (Figure 3A). However, inhibition of H3K27me3 by knockdown of PRC2 components EED, EZH2, or SUZ12 decreased iPSC reprogramming, potentially due to a failure to silence fibroblast specific transcripts which gain H3K27me3 during successful iPSC reprogramming [11] (Figure 3A). Disruption of MBD3 or GATAD2A in the NuRD complex, which normally facilitates repression through histone de-acetylation and remodeling, enhanced iPSC reprogramming [95]. GATAD2A siRNA knockdown was

also shown to improve the activation of genes located in H3K27me3 heterochromatin during fibroblast to hepatocyte reprogramming [10], potentially due to decreased H3K27 de-acetylation [95]. Gene de-repression alone is typically not sufficient for activation during reprogramming which requires both de-repression as well as the presence of an activating transcription factor [10,15].

The rationale here is that a transient diminution of heterochromatin proteins can allow the reprogramming factors to activate new genetic networks, and the subsequent restoration of heterochromatin proteins, after transient diminution, can allow a new genetic network to re-establish heterochromatin appropriate for the new cell type.

However, such manipulations can be a dangerous game. Heterochromatin opening during reprogramming can lead to activation of off-target lineages and repeat elements [10]. To lessen this problem, recent findings reveal that groups of heterochromatin proteins co-repress distinct sets of genes located in heterochromatin and each gene set possesses a particular chromatin signature [10]. While H3K9me3 HMTs and complexes such as HUSH target H3K9me3 to broad classes of genes and repeat elements for repression, recent findings have identified heterochromatin proteins necessary for subsets of H3K9me3 targets (Figure 3B). Thus, to more precisely open heterochromatin domains and lessen the undesired consequences, it is necessary to learn more about the mechanisms by which the heterochromatin machinery is targeted in a locus- and gene-specific manner.

Recently it was demonstrated that depletion of Enhancer of Rudimentary Homolog (ERH) in human cells, the *S. pombe* homolog of which is a known regulator of H3K9 methylation [96,97], leads to global H3K9me3 loss in human cells, activation of heterochromatic protein coding genes during induced hepatocyte reprogramming, and activation of satellite repeats [10] (Figure 3A). In *S. pombe*, Erh1 interacts with the YTH domain-containing protein Mmi1 and is recruited in an RNA-dependent manner to meiotic genes, to maintain H3K9me3 heterochromatin and silencing [96,97]. Despite a conserved protein sequence with Erh1 [96] and H3K9me3 regulatory function [10], the mechanism of ERH recruitment in humans is unknown, as the direct ortholog of Mmi1 is absent in mammals [98]. Surprisingly human ERH was found to repress genes in heterochromatic and euchromatic H3K9me3 domains, indicating that it may function in the targeting of many or most of H3K9me3 deposition mechanisms [10].

Although heterochromatin is partially defined by its transcriptionally silent nature, recent findings demonstrate a role for RNAs in heterochromatin establishment and maintenance, beyond the canonical role of the XIST RNA in X inactivation [37]. RNA-directed heterochromatin establishment is of particular interest due to the potential for uncovering target specificity, which could allow specific RNAs to be disrupted to unlock specific heterochromatin domains. An example of such sequence specificity can be observed in RNAi-directed post-transcriptional gene silencing by which nuclear Argonaute proteins establish repression in *S. pombe, D. melanogaster, A. thaliana* and *C. elegans* [22,41], nuclear Argonaute proteins in mammals, however, may be involved in both activation and repression [99].

Euchromatic H3K9me3 regions are transcriptionally dampened but not fully silenced by the HUSH complex, which recruits SETDB1 to repress evolutionarily young L1 retrotransposons, naïvely integrated lentiviruses, and tissue specific genes including ZNF gene clusters [100]. The HUSH complex is recruited by intronless RNAs, a feature of retroelements, to repress transgenes and mobile elements [101]. In turn, the repression by HUSH also produces shorter non-polyadenylated transcripts, favorable for nuclear exosome targeting (NEXT) degradation [102]. HUSH complex suppression of L1 elements is required for self-renewal of ground-state pluripotent stem cells [103], but depletion of HUSH complex component Periphilin 1 enhanced activation of genes in heterochromatin during reprogramming to hepatocytes [10]. How HUSH is targeted to genes with introns such as the ZNF clusters remains unclear.

In parallel, heterochromatic H3K9me3 domains are highly enriched for HP1 proteins, which can bind RNA through HP1's hinge domain [62]. Recent in vitro modeling suggests that the affinities of IAP and satellite RNAs for HP1 proteins are five-fold higher than for Mediator complexes, therefore partially explaining the different recruitment mechanisms to repeats versus gene promoters and enhancers [104]. Depletion of HP1 proteins during reprogramming destabilizes H3K9me3 heterochromatin domains that repress pluripotency genes, and therefore enhances reprogramming efficiency [105]. SAFB, a nuclear matrix associated protein, binds major satellite RNA to promote phase separation at the boundaries of H3K9me3 marked heterochromatin domains [106]. Interestingly SAFB has been demonstrated to interact with ERH [107] and may cooperate in miRNA processing [108]. Deletion of YTHDC1, which targets the RNA modification m^6A to direct SETDB1 H3K9me3 to retrotransposons and totipotent genes, in mouse ESCs initiated reprogramming to a 2-cell like totipotent state [55] (Figure 3A).

Heterochromatin opening can be facilitated by the active removal of repressive marks and addition of activating marks to histones. Ectopic lysine demethylases, KDM6A and KDM4B, targeting to H3K27me3 and H3K9me3 domains, respectively, improved reprogramming [109,110] (Figure 3A). Similarly, increased histone acetylation, triggered through pathways downstream of MAP2K6 phosphorylation, can lead to improvements in Sox2 and Klf4 binding and reprogramming to pluripotency [111].

These studies reveal that heterochromatin de-repression can be triggered by disrupting maintenance functions or active heterochromatin removal, making target chromatin more permissible and improving reprogramming by transcription factors.

Selectively de-repressing heterochromatin domains

The activation of unintended transcripts, including repeat elements and alternative lineage genes [10], as well as increased genome instability associated with widespread heterochromatin de-repression [17,93,112], remains a major barrier to heterochromatin diminution for cell therapy applications. The goal remains to selectively de-repress specific heterochromatic gene sets or domains while maintaining repression of repeat regions and undesired genes. Further work to understanding how the HUSH complex [100–102], ERH [10,96], or YTHDC1 [55] are recruited or maintained in chromatin will be key. It

is important to note that, as best we understand, disrupting H3K9me3 heterochromatin maintenance still requires either dilution through cell division [113] or the action of demethylases, for the H3K9me3 mark to go away [114]. Understanding which H3K9me3 HMTs are targeted and how this targeting can be disrupted is complicated by their ability to function redundantly and compensate for partial losses of the other of the three H3K9me3 HMTs [13,22,93]. Another approach involves the identification of highly specific repressors, such as sequence specific ZNFs [71–74], or the design of synthetic de-repressors, which has been done recently by fusing epigenetic regulators to transcription activator-like effectors [112] and dCas9 [115].

The transcriptional outcome of derepressing H3K9me3 domains may be influenced by other marks that are either coincident with H3K9me3 or that are established in a compensatory manner. For example, in mouse ESCs, dual H3K36me3/H3K9me3 domains, but not H3K9me3-only domains, gained interaction with upregulated genes upon a SETDB1 knockout [116]. H3K9me2 [15] and compensation by H3K27me3 [10,13,93] have been shown to maintain repression at a subset of sites after loss of H3K9me3. Better understanding the complex landscape of heterochromatin will be key to enabling precise and selective de-repression.

Concluding Remarks

Despite the extensive rearrangement of heterochromatin during development, genome stability and repression of repeats are maintained, indicating that different types of heterochromatin can be selectively modulated. Different heterochromatin complexes, directed by RNA and transcription factors, appear at different chromatin domains to accommodate various developmental needs. By discovering the mechanisms by which heterochromatin is selectively targeted during development, we hope to selectively derepress key genes in heterochromatin for reprogramming to diverse cell types, without activating repetitive regions and off-target genes that are seen with global heterochromatin loss [10,93]. Recent advances in human iPSC reprogramming suggests that the route(s) to pluripotency transiently goes through a totipotent state [117], which was recently captured in vitro [118], offering an opportunity to reconstitute early human development in vitro and investigate the heterochromatin remodeling underlying cell fate transitions in greater detail. Future studies are required to dissect upstream signaling pathways, and examine the functional consequences of disrupting different heterochromatin associated proteins and complexes in various developmental and reprogramming contexts to establish a more unifying principle that governs heterochromatin functions (see Outstanding Questions). Finally, understanding how pioneering factors interact with silenced chromatin will also inspire novel designs for synthetic reprogramming factors that combine the chromatin binding capacities of pioneer factors with chromatin effector domains that modulate repressive heterochromatin environments to improve reprogramming.

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Outstanding Questions Box

- How are heterochromatin proteins dynamic at particular genomic locations in a cell type specific manner, e.g., during development?
- What are the roles of RNA binding proteins and RNAs in regulating heterochromatin at lineage specific genes and how they can be targeted to enhance reprogramming?
- How can heterochromatin at genes be de-repressed while maintaining repression of repeats and transposable elements?
- How can manipulating heterochromatin be used to improve cellular reprogramming?

Highlights

- Various categories of heterochromatin exist and are regulated by different proteins, RNAs, and mechanisms to restrict access by transcription factors in different ways and degrees.
- Activation of a gene during reprogramming that was in heterochromatin requires both the opening of the heterochromatin and activating factors.
- Disrupting mechanisms required for maintenance of heterochromatin makes sites permissive to transcription factor binding and activation, but can cause activation of off-target genes and repeat elements.

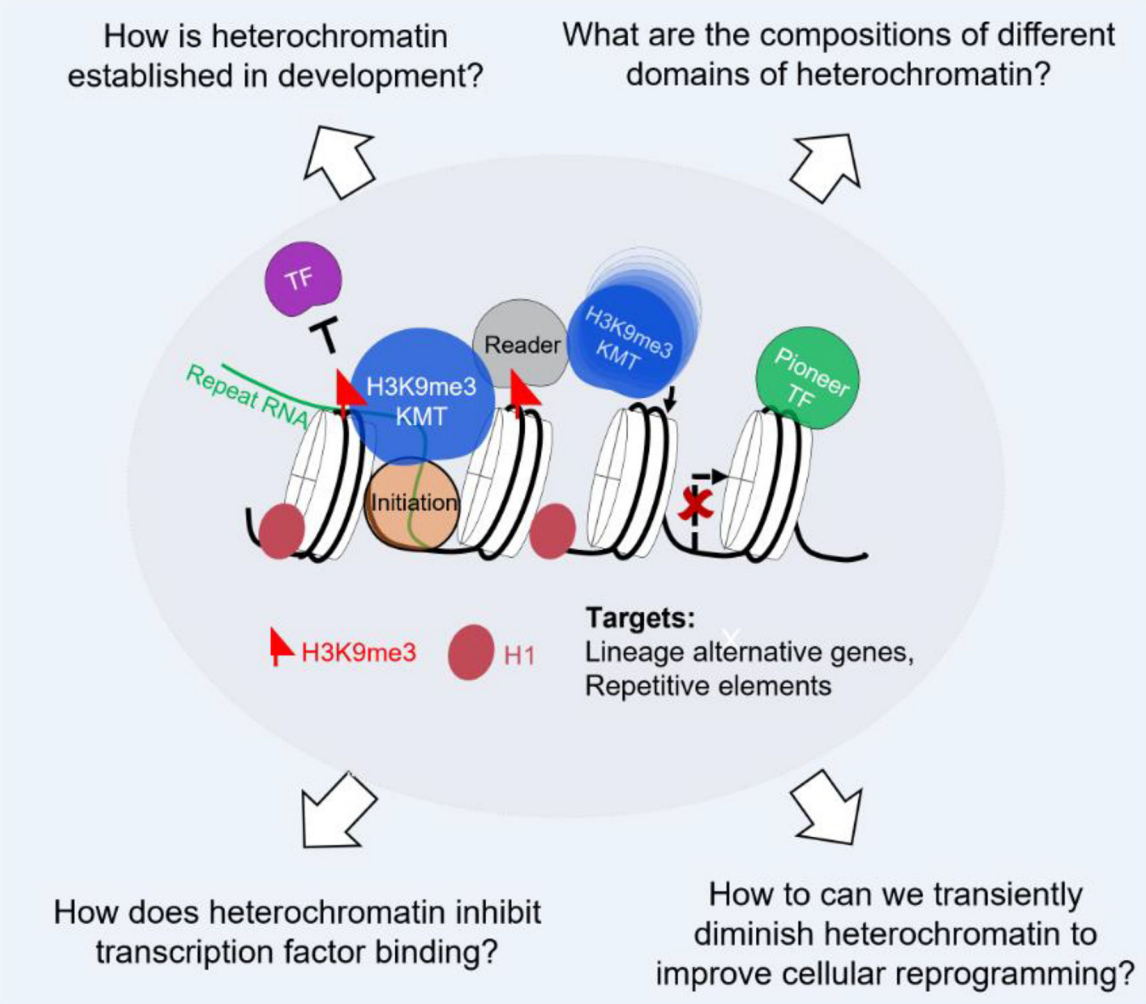

Figure 1.

H3K9me3-heterochromatin as a barrier to cell fate change. Central to the functions of H3K9me3-heterochromatin is the “reader-writer” module, in which H3K9me3 mark deposited by H3K9me3 methyltransferases is recognized by reader proteins, including HP1α/β/γ, which further recruit methyltransferases to modify the neighboring nucleosomes. This leads to spreading of heterochromatin domains and stable maintenance of H3K9me3 domains over the cell cycle. Further enrichment of linker histone H1, HP1 proteins and other heterochromatin associated proteins lead to heterochromatin compaction and restricting the TFs from activating their targets. Building from this basic principle, we discussed how heterochromatin is established and maintained in development, different compositions of heterochromatin domains, how it molds the TF bindings and finally how to this knowledge to enhance cellular reprogramming.

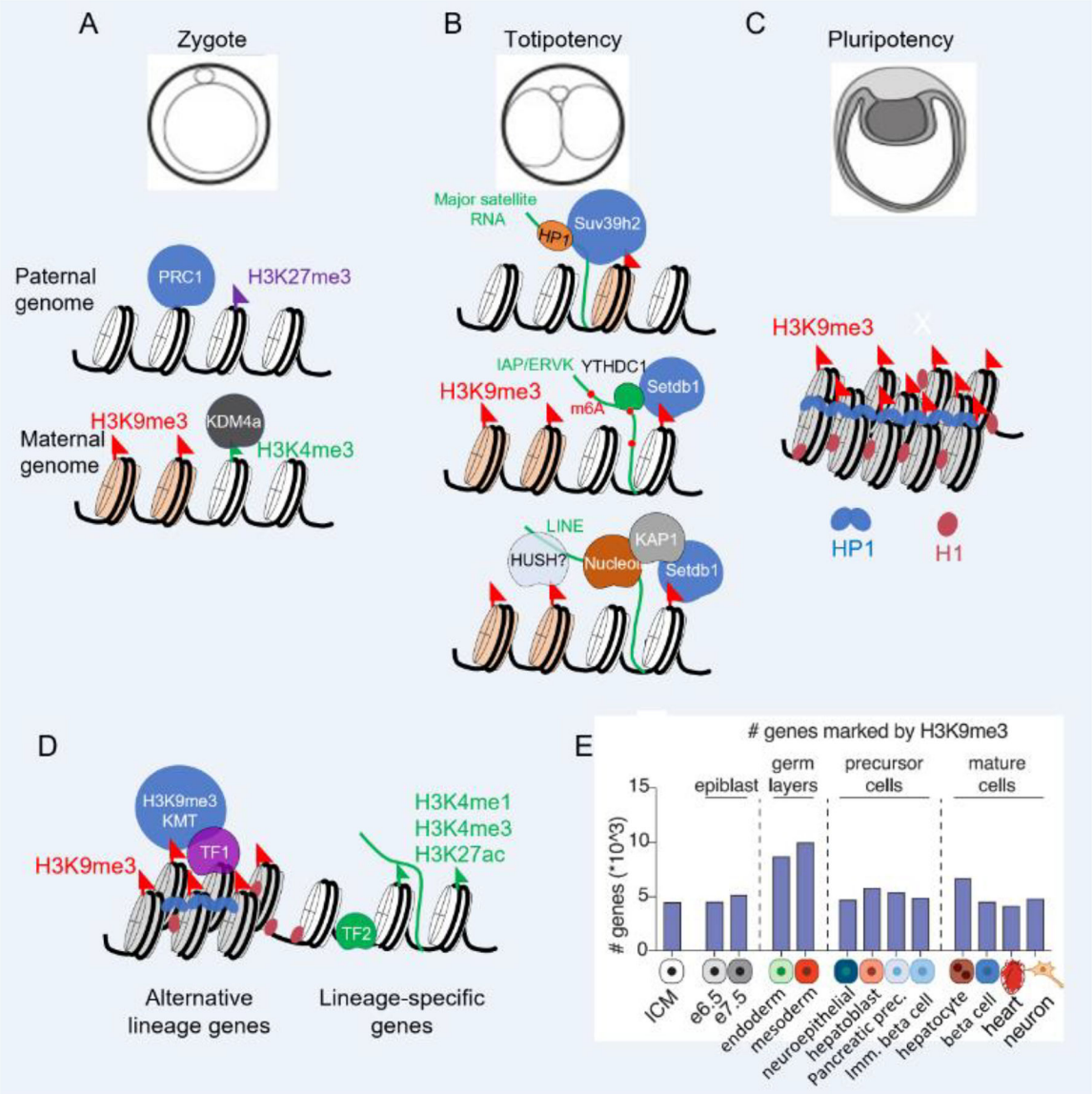

Figure 2.

Heterochromatin is dynamic during development. (A) Heterochromatin remodeling accompanies developmental progression during early mouse development. In the zygote, the maternal genome possesses H3K9me3 heterochromatin marks at centromeric and pericentromeric regions, whereas paternal genome does not. (B) An ensuing heterochromatin remodeling creates an open chromatin environment, a hallmark of totipotent states and leads to activation of repeat regions, which recruit heterochromatin machinery to establish heterochromatin and promote the transition from totipotency to pluripotency. (C) Heterochromatin domains in pluripotent stem cells are decorated with H3K9me3 marks, compacted by linker histone H1 and recruit heterochromatin associated proteins, including HP1. (D) During lineage specifications in mouse development, transcription factors, including KRAB-ZNF proteins direct heterochromatin machinery to repress alternative lineage-specific genes to maintain the cell fate. (E) Genes are increasingly

marked by H3K9me3 for repression during germ layer development, but this mark is removed from key functional genes upon lineage specification [13].

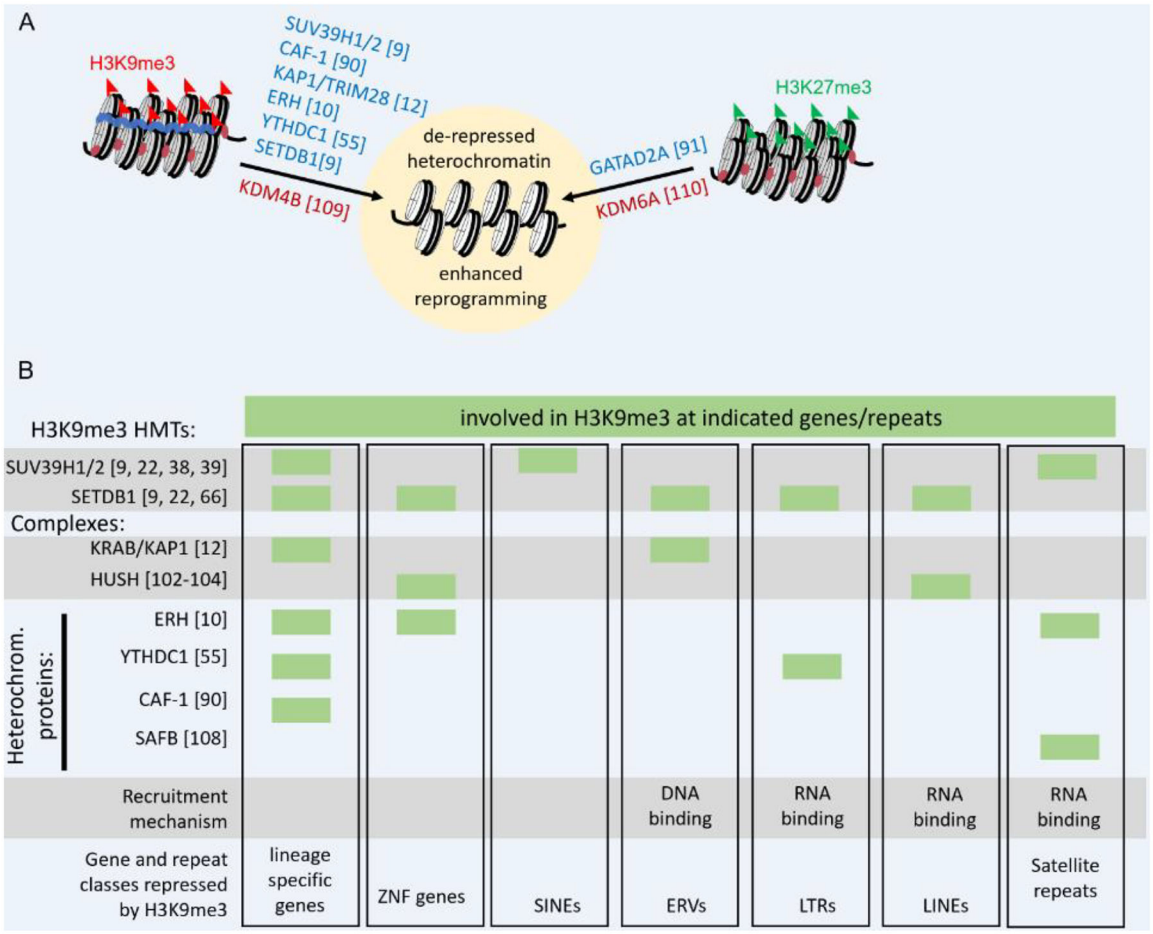

Figure 3.

Groups of heterochromatin proteins regulate distinct classes of heterochromatin and can be disrupted to facilitate gene activation. (A) Published results showing knockdowns/knockouts (blue) and overexpression (red) experiments that lead to de-repression of heterochromatin and enhanced reprogramming. (B) Regulation of H3K9me3 at genes and repeat classes by H3K9me3 HMTs, protein complexes and selected heterochromatin proteins. Green boxes indicate the indicated protein or complex has been experimentally demonstrated to regulate H3K9me3 at the designated gene or repeat class.