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The Yin and Yang of chromatin dynamics in adult stem cell fate selection

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Abstract

Adult organisms rely on tissue stem cells for maintenance and repair. During homeostasis, the concerted action of local niche signals and epigenetic regulators establish stable gene expression patterns to ensure that stem cells are not lost over time. However, stem cells also provide host tissues with a remarkable plasticity to respond to perturbations. How adult stem cells choose and acquire new fates is unknown, but the genome-wide mapping of epigenetic landscapes suggests a critical role for chromatin remodeling in these processes. Here, we explore the emerging role of chromatin modifiers and pioneer transcription factors in adult stem cell fate decisions and plasticity, which ensure that selective lineage choices are only made when environmentally cued.

Keywords

chromatin dynamics; adult stem cells; cell identity; fate selection; plasticity; super-enhancer

Adult stem cells in and out of their niche

One of the fascinating questions in developmental biology is how different cells within a given tissue acquire different properties that allow them to work together as a functional unit. At the heart of this problem are adult stem cells, which are essential building blocks to fuel tissue homeostasis and regeneration. Adult stem cells reside in specialized niches, which profoundly impact their activity and maintenance. Changes in this microenvironment allow stem cells to exit the niche and make tissue and/or repair wounds. However, this poses a dilemma for stem cells: how can they retain their ability to survive outside the niche, how do they choose appropriate fates and what defines the point of reversibility versus commitment to differentiation? The notion that stem cells acquire greater fate flexibility after injury or transplantation suggests that besides the impact imposed on stem cells by their native niche, additional mechanisms must be in place to govern stem cell identity, fate decisions and plasticity (see glossary) (Box 1).

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While genome-wide chromatin mapping of cultured embryonic stem cells and other cell types have provided new insights into cellular states, *in vitro* mRNA and protein expression profiles have long been known to differ quite dramatically from their *in vivo* tissue counterparts. Such observations suggest that gene expression, and likely chromatin dynamics, of stem cells will also be highly dependent upon their native niche microenvironment. If so, tackling the mechanisms underlying chromatin dynamics and their physiological relevance will necessitate *in vivo* analyses. This is especially important for adult stem cells, where there are often multiple steps in lineage commitment that cannot be easily understood or recapitulated outside the confines of the tissue. Indeed, even with the handful of recent *in vivo* studies conducted thus far, it is already clear that cell-intrinsic, dynamic chromatin modifications play major roles in adult stem cells, which make lineage choices by integrating changes in niche signals with transcriptional circuitries that determine cell identity.

In this review, we focus on various adult stem cell populations and summarize recent advances on chromatin dynamics that have contributed to the emergence of new concepts in stem cell biology.

DNA methylation – no longer just a stable silencing mark

Although the full complexity of epigenetic regulation is only starting to unveil, DNA methylation is of particular relevance for tissue homeostasis. DNA methylation provides a means for functional variability while maintaining the information content of the nucleotide: In mammals, the fifth carbon of the pyrimidine ring of CpG dinucleotides can become methylated (5mC) [1]. Due to the spontaneous deamination of 5mC, C→T transitions at CpG dinucleotides account for >30% of all point mutations in human genetic disorders.

During development, CpG methylation is established by *de novo* DNA methyltransferases DNMT3A and DNMT3B [2]. The 5mC pattern is then faithfully preserved by DNMT1, which is targeted to hemimethylated DNA by UHRF1 during DNA replication [3]. While the majority of cytosine residues within CpG dinucleotides are methylated, CpG islands at promoters remain mostly unmethylated, a feature that has long been surmised to create a permissive environment for transcription initiation [4]. Indeed historically, DNA methylation has been considered a stable silencing mark, ensuring tissue-specific gene expression in a heritable manner throughout development. As such, DNA methylation is critical for control of gene transcription, establishment of cellular identity, silencing of transposon elements, parental imprinting and X-chromosome inactivation [2].

The presence of 5mC is thought to inhibit transcriptional activation by preventing the binding of many transcription factors to DNA and by recruitment of methyl-binding proteins (e.g. MeCP2 or MDB1) and histone deacetylases, which ultimately generate a repressed chromatin environment [5]. However, recent evidence suggests that DNA methylation is more dynamic than hitherto appreciated. Although 5mC can be lost passively through imperfect maintenance, the discovery of ten-eleven translocation (TET) family enzymes provided a compelling means for catalyzed active demethylation [6]. TET enzymes first convert 5mC into 5-hydroxymethylcytosine (5hmC), which can subsequently be reverted to

cytosine through iterative oxidation and thymine DNA glycosylase (TDG)-mediated base excision repair [7-9].

Dynamic DNA methylation is achieved by the interplay between DNMT and TET enzymes, and becomes a powerful strategy to regulate gene activity, as was recently found for stem cell lineage progression. Even though global changes are modest, dynamic DNA methylation/demethylation in adult stem cells occurs specifically in regulatory regions of developmental genes and correlates with the presence of cell-type specific transcription factors [10,11]. Hence, differentiation-associated gene promoters are frequently methylated in blood or skin stem cells, yet lose this repressive mark upon fate commitment. Concomitantly, regulatory elements of other lineages become increasingly methylated to lock cells in their differentiated fate [12-14].

Although DNA methyltransferases are dispensable for self-renewal of embryonic stem cells [15], DNMT1 is essential for the maintenance of epithelial [16-18], mesenchymal [19] and hematopoietic stem cells [20,21]. Depletion of DNMT1 from skin stem cells causes aberrant differentiation due to marked de-repression of epidermal differentiation genes [16,17]. Mice conditionally targeted for *Dnmt1* also exhibit progressive alopecia owing to self-renewal defects and increased apoptosis in hair follicle stem cells [17]. *Dnmt1*-null hematopoietic stem cells similarly show premature exhaustion [20,21]. Despite global hypomethylation, DNMT1-deficient hematopoietic stem cells selectively induce myeloid-progenitor transcription factors and display a skewed distribution towards myeloid fates, at the expense of the lymphoid lineage [20]. In contrast, loss of *de novo* DNA methyltransferase DNMT3A results in hematopoietic stem cell expansion and impaired differentiation [22]. Combined loss of DNMT3A/DNMT3B is synergistic, resulting in enhanced hematopoietic stem cell self-renewal and greatly exacerbated defects in differentiation [23]. Collectively, these studies indicate that DNA methyltransferases have distinct functions in adult stem cells. DNMT1 may serve to balance alternative fates by protecting stem cells from premature activation of predominant differentiation programs, whereas DNMT3A/B appear to license the differentiation program.

The impact of DNMTs on adult stem cell lineage progression has long been attributed to a generally repressive function of DNA methylation, irrespective of position and density of CpG motifs [4]. Recent evidence adds new dimensions to this traditional view. Similar to hematopoiesis, neurogenesis critically depends on DNMT3A, which exerts a dual role in neural stem cells [24]. On the one hand, DNMT3A represses glial-differentiation genes by methylating CpG islands. On the other hand, it promotes the expression of neurogenic genes by functionally antagonizing Polycomb-mediated gene silencing by methylating non-proximal promoter elements, including gene bodies [24]. Indeed, recent advances in genome-scale mapping have demonstrated that intragenic methylation is quite prevalent in actively transcribed genes, where it is thought to promote transcriptional elongation, suppress alternative promoters and potentially influence splicing [25-27]. *De novo* methylation of active genes is coupled with H3K36me3, a histone modification associated with transcriptional elongation, which can directly be recognized by DNMT3B via its PWWP domain [28]. These findings have nurtured our understanding that the impact of DNA methylation on transcriptional activity is more nuanced than previously appreciated.

TET enzymes: novel epigenetic regulators in stem cell lineage progression

DNA methylation must be dynamic to ensure tissue homeostasis. The discovery of TET enzymes provided the long-sought demethylases that remove CpG methylation marks on DNA [6-9]. The specific enrichment of 5hmC in mammalian brain suggests that TET-mediated hydroxymethylation, providing an intermediate step in DNA demethylation, is particularly critical for neuronal development and brain function: When *Tet1* is conditionally targeted for ablation in mice, neural stem cell expansion is impaired due to promoter hypermethylation of pro-proliferative genes, which in turn leads to impaired hippocampal neurogenesis and cognitive disorders [29].

In contrast, TET2-deficiency in the hematopoietic lineage increases stem cell self-renewal, endowing them with a competitive advantage. The outcome is reduced lineage progression and accumulation of premalignant clones [30-34]. Despite the seemingly opposing actions of TET1/2 in different stem cell compartments, these results suggest that tissue-specific demethylation is indispensable for stem cells to embark upon cellular differentiation programs. However, specific 5hmC readers have now been identified, suggesting that 5hmC might be more than a de-methylation intermediate but also an epigenetic regulator itself [35,36]. 5hmC is also enriched at lineage-specific enhancers, which are dynamic and frequently become demethylated upon gene activation [60].

Although these studies have established a critical role for dynamic DNA methylation in adult stem cells, there is a relative paucity of CpG in the mammalian genome: the majority of CpG islands remain unmethylated during development and stem cell differentiation [1,2]. If DNA methylation alone regulated chromatin dynamics, there would be a more permissive transcriptional environment than the <50% of genes that are actively expressed in any mammalian cell type. In reality, many genes with unmethylated CpG islands are inactive, either due to a lack of the appropriate transcription factors, or the existence of other epigenetic mechanisms, such as the Polycomb complex, that achieve chromatin silencing [1].

Polycomb-repressive complexes as safeguards against impromptu lineage choice

Polycomb-group (PcG) proteins form multi-subunit chromatin remodeling complexes known as Polycomb repressive complexes (PRC) that orchestrate the sequential modification and compaction of chromatin, resulting in potent repression of target genes [37].

The mammalian PRC2 complex consists of EZH2, EED, SUZ12 and RBAP48 subunits and is the first player to be recruited to chromatin. The methyltransferase EZH2 then catalyzes the tri-methylation of histone H3 lysine 27 (H3K27me3) at target gene promoters, which is associated with transcriptional repression [37]. Subsequently, the PRC1 complex, comprising CBX, RING1A/B, PH and BMI1 subunits, deposits a mono-ubiquitination mark on histone H2A lysine 119, a modification that impedes RNA polymerase II elongation and promotes chromatin compaction and silencing [37]. The ability of PRC1 subunit CBX to

recognize H3K27me3 facilitates the concerted action of PRC2/1 and thus warrants potent gene silencing, although variant PRC1 complexes can be recruited to chromatin and initiate silencing in the absence of PRC2 (Figure 1, Key Figure) [38,39].

Since PRC2 is recruited to a large cohort of developmental genes in embryonic stem cells, it has been proposed that PcG-silencing is critical to regulate lineage fidelity by preventing unscheduled induction of non-lineage and differentiation genes. Histone methylation profiles in adult stem cells further support this notion. In skin stem cells, genes associated with stemness are decorated with activating H3K4me3 and H3K79me2 marks, while non-epidermal and differentiation genes are H3K27me3-repressed. Conversely, committed short-lived progeny acquire high levels of H3K27me3 on stemness genes, while differentiation genes lose the repressive mark (Figure 1, Key Figure) [40]. This indicates that, in addition to DNA methylation changes, stem cell lineage progression is governed by the dynamic regulation of PcG-silencing. In contrast to embryonic stem cells, where key developmental genes exist in a bivalent state and display both activating H3K4me3 and PcG-repressive H3K27me3 marks, adult stem cells have very few genes exhibiting this ‘poised’ behavior [40]. Thus, bivalency may be less critical once tissue lineages have been selected and cell type options have been restricted.

The expression of PcG proteins highly correlates with developmental stage. Several PRC1/2 subunits are robustly expressed in epidermal and neural progenitors, but their expression wanes in committed cells [41,42], which cease proliferation and switch to a program of differentiation. However, fate commitment requires relief of PcG-repression on differentiation gene promoters, a process that depends on the H3K27me3 demethylase JMJD3 [43,44]. A similar switch is observed during tissue regeneration after injury, which relies on stem cells and their ability to choose appropriate fates. Upon skin wounding, PRC subunits are repressed, whereas JMJD3 is upregulated at the wound site as the genes needed for repair are rapidly induced [45].

Genetic studies have substantiated these roles of PcG-silencing in governing stem cell function. Although loss of EZH2 in embryonic stem cells reduces global H3K27-methylation levels, H3K27me3 on key developmental genes is surprisingly intact. This has been attributed to the activity of EZH1, which can functionally compensate for EZH2 in embryonic stem cells [46]. Evidence from adult stem cells further support the notion that alternative PRC2 complexes have evolved as safeguards to reinforce cellular identity in mammalian organisms [47,50]. The activation of aberrant lineage fate genes that occurs upon EZH1/2 loss of function is not nearly as high as that normally seen within the proper lineage itself, revealing the combined role of lineage-specific transcription factors and relief of PcG-repression in regulating fate determination [47].

In embryonic epidermis, EZH2 controls the proliferative potential of basal progenitors by preventing the premature recruitment of AP1 transcription factors to genes required for epidermal differentiation [41]. Intriguingly in adult mice, *Ezh2* deletion has no apparent consequence to skin integrity. In contrast, complete loss of PRC2 function by *Ezh1/2* double conditional knockout in skin abolishes the H3K27me3 mark and severely compromises hair follicle formation and maintenance [47,48]. Although hair follicle stem cell specification is

unaffected, PRC2 loss of function specifically affects stem cell self-renewal and hence tissue regeneration and wound repair. Deletion of EZH2 in blood progenitors similarly compromises fetal liver hematopoiesis but not the self-renewal capacity of adult hematopoietic stem cells in bone marrow [49]. Instead, adult hematopoietic stem cells rely more on EZH1, which promotes a slow cycling state and prevents differentiation and senescence [50]. Insights obtained from EZH2-deficient neural and muscle stem cells further support the critical role of PcG-repression in stem cell self-renewal [51,52].

Mechanistically, the inability of PcG-deficient adult stem cells to proliferate is deeply rooted in untimely activation of the *Ink4a/Arf/Ink4b* locus [47,50-52], which encodes tumor-suppressor proteins that arrest the cell cycle. Genetic deletion or shRNA-mediated suppression of the *Ink4a/Arf* locus restored the proliferation and survival of adult stem cells defective in PcG components [47,50,51]. While these studies have exposed the sensitivity of the *Ink4a/Arf/Ink4b* locus to PcG-repression, it is noteworthy that unlike in embryonic stem cells [53], the global loss of H3K27me3 does not result in widespread differentiation defects. Even though PcG-silenced genes are transcriptionally activated in hair follicle stem cells in the absence of EZH1/2, the amplitude of non-epidermal/hair follicle differentiation programs are too low to overcome already established stem cell fates [40,47]. Similarly, stable knockdown of SUZ12, a core subunit critical for PRC2 assembly and function, leads to precocious activation of terminal differentiation markers, but is insufficient to cause phenotypic fate switches in intestinal stem cells [54]. Thus, even though PcG-silencing places a molecular threshold on the irreversible transition from a multipotent activated stem cell to a differentiation-committed cell, the absence of phenotypic fate switches in PcG-deficient stem cells underscore the importance of additional epigenetic modifiers that have evolved to ensure the reinforcement and maintenance of lineage programs in adult tissues. Indeed, adult stem cells seem to couple PcG-silencing with a requirement for cell-type specific transcriptional activators, to ensure that cell identity is robustly maintained during homeostasis and that selective lineage choices can only be made when environmentally cued.

Lineage-specific enhancer selection by pioneer transcription factors

Transcription factors shape the precise gene expression pattern required for a cell to perform its unique functions. They dictate cell fates by activating genes critical for the identity of particular cells while concurrently antagonizing lineage-inappropriate genes [55-58]. Genome-wide studies have demonstrated that the majority of transcription factor binding sites are located in distal enhancers [59]. Mechanistically, transcription factor binding at enhancers activates a cascade of epigenetic events initiating from recruitment of transcriptional co-activators, to covalent modification (e.g. methylation and acetylation) of histone tails, loss of 5mC levels, and culminating in increased transcriptional output (Figure 1, Key Figure) [59,60]. Thus, enhancers are characterized by nucleosomal depletion, DNaseI hypersensitivity and enrichment of histone acetyltransferase p300/CBP occupancy, suggesting an overall open chromatin structure [59].

Although active promoters and enhancers typically possess H3K27ac, enhancers are characteristically enriched in H3K4me1, in contrast to H3K4me3 found at promoters

[61-64]. Therefore, enhancers can readily be distinguished from promoters by their unique epigenetic modification signatures. That said, recent evidence suggests that promoters and enhancers share common sequence structures and operational characteristics. Enhancers are also enriched for RNA Polymerase II, resulting in active transcription of enhancer-associated RNAs (eRNAs) [59].

Although mammalian genomes contain a vast number of putative enhancers, only a fraction is active within a given cell, raising the question how unique enhancer repertoires are selected. Earlier studies suggest that cell-type specific enhancer selection involves the binding of lineage-determining transcription factors that “prime” enhancers. These so-called ‘pioneer factors’ have the unique ability to engage target sites in condensed chromatin, upon which they trigger nucleosomal remodeling to grant chromatin access to additional factors [65]. As such, pioneer factor binding occurs prior to lineage commitment and employs a chromatin-opening step to establish competence for gene activation. However, pioneer factor binding is also dependent on the chromatin environment. Although certain pioneer factors can bind methylated DNA, H3K9me3 heterochromatic domains represent a barrier and may provide a means for cells to stably retain their fate [66]. Thus, histone/DNA modifications and pioneer factors are likely to function synergistically to establish competence for transcription. Well-characterized pioneer factors include forkhead box A (FOXA) factors, GATA-binding (GATA) factors and PU.1 [65], and a recent study established a pioneer factor role for pluripotency factors OCT4, SOX2 and KLF4 [67].

However, most transcription factors, including pioneer factors, are not lineage-specific *per se*. Despite their broader expression pattern, transcription factors may exhibit entirely different binding patterns and regulate non-overlapping sets of target genes in different cell types and tissues [68-71], because they often bind cooperatively to DNA as multi-protein complexes, whose composition is typically cell-type specific [72-75]. On the flip side, chromatin accessibility, DNA methylation patterns and enhancer usage are highly variable across different cell types [76,77]. As a consequence, cellular identity is the result of the combinatorial action of distinct transcription factor complexes on lineage- and cell-type-specific enhancers.

Transcriptional circuitries within Super-enhancers govern cellular identity

Recent evidence suggests that genes specifying cell identity and function are associated with densely spaced clusters of active enhancers referred to as ‘super-enhancers’, which recruit much of the cell’s transcriptional apparatus (Box 2) [78-80]. Although small in number (<5% of total enhancers), super-enhancers encompass large open chromatin domains richly decorated with H3K27ac modifications and abundant in cell-type and identity-specific transcription factor binding motifs that enable transcription factors to bind cooperatively [78]. The wiring of cell-type specific transcription factors as self-reinforcing circuits within super-enhancers allows for robust maintenance of specific transcriptional landscapes and thus governs cell identity.

Initially identified in embryonic stem cells [78], a recent study highlights that super-enhancers underlie the identity, lineage progression and plasticity of adult stem cells *in vivo*

[81]. Stem cell identity in the hair follicle niche depends on a cohort of transcription factors [82-90]. *In vivo* ChIP-seq analysis has shown that these transcription factors bind cooperatively to dense clusters ('epicenters') within hair follicle stem cell super-enhancers to regulate ~400 putative stemness genes, including themselves (Box 2) [81]. Intriguingly, super-enhancers and their epicenters are cell-stage specific and change dramatically upon lineage progression, wounding or transplantation. Once triggered by external factors, new fate is acquired by decommissioning old and establishing new super-enhancers, an auto-regulatory process that involves Polycomb-silencing of one transcription factor cohort while super-enhancer-activating another [81]. Thus, by coupling bidirectional chromatin switches with antagonistic relationships between transcription factor sets governing alternate cell fates, stem cells establish barriers to cellular plasticity and provide a basis for their mutual exclusivity during fate commitment.

Stem cell behavior and plasticity depends on their microenvironment

Dynamic chromatin remodeling need not always be associated with fate change. When outside their native niche, stem cells can maintain their identity and yet must dramatically change their chromatin landscape to adapt and survive. In culture, hair follicle stem cells extensively remodel super-enhancers and suppress master identity genes, including hair follicle stem cell-specific transcription factors. However, upon engraftment after passage in culture, these cells reorganize a new niche and regain expression of super-enhancer-regulated hair follicle stem cell transcription factors. Intriguingly, many of the changes arising in cultured hair follicle stem cells are also induced after wounding [81]. Similar features of plasticity were recently reported for tissue-resident macrophages, which display specific enhancer signatures associated with distinct combinations of transcription factors that are dynamic and responsive to local microenvironments [91,92]. These findings emphasize that cells are highly sensitive and aware of their microenvironment, and provide an explanation for how stem cells can maintain a reversible, yet plastic state when outside their niche. However, chromatin remodeling in tune with the microenvironment poses a dilemma: How can cells retain their unique identities in the face of dramatically altered transcriptional landscapes in different microenvironments? For stem cells, this feature is particularly relevant, since they not only have to survive outside their niche, but also retain stemness.

In the hair follicle, this is achieved through modulation of SOX9 levels, which acts as pioneer regulator of stem cell plasticity. Although SOX9 is retained at low levels in culture, it is essential: Upon ablation, cultured stem cells are lost, while SOX9 overexpression restores the cohort of hair follicle stem cell factors *in vitro*, similar to what happens upon engraftment and reestablishment of the niche. Forced expression of SOX9 in epidermis, where it is typically absent, activates PcG-repressed super-enhancers of other hair follicle stem cell transcription factor genes. When SOX9 is sustained during stem cell commitment, all hair follicle stem cell transcription factors remain high and hair differentiation is impaired [81]. This exemplifies that compared to loss of PcG-repression, sustained super-enhancer activation appears more potent in governing lineage switches.

Home sweet home: the impact of niche signals

Following the loss of stem cells in skin or the intestine, early stem cell progeny can readily return to the niche and revert back to being stem cells [93-97] (Box 1). By contrast, when niche components are ablated, stem cells cannot respond to tissue-regeneration cues [98], underscoring the importance of the niche in dictating stem cell behavior [99-102]. Therefore, epithelial stemness is not only an inherent feature of particular cells, but can be induced by niche signals if the chromatin landscape is permissive and master regulators are retained. Indeed, intestinal crypts exhibit broadly permissive chromatin, as enhancers for both absorptive and secretory lineages show signs of activation in undifferentiated stem cells and both classes of progenitors [103]. Even though some enhancers are similarly preset in hematopoietic stem cells, stepwise analysis indicated that the chromatin landscape of hematopoietic stem cells is dynamically reorganized at each point of lineage divergence [104,105].

Yet what are the external signals that promote dynamic chromatin rearrangements to induce stem cell lineage commitment? Similarly, what dictates the levels of pioneer factors that endow stem cells with features of plasticity? Although the evidence for adult stem cells is sparse, studies in cultured embryonic stem cells suggest that super-enhancers are particularly enriched for binding sites of terminal transcription factors of developmental signaling pathways, including WNT, TGF-beta or LIF/STAT3 [106]. Super-enhancers in hair follicle stem cells similarly show a dramatic enrichment for WNT effectors TCF3/4 [81]. As such, it is feasible that super-enhancers provide a platform for signaling pathways to coordinate stem cell behavior with their microenvironment. Thus, if changes in niche signals reach a certain threshold, chromatin remodeling of super-enhancers may be sufficient to push stem cells beyond the point of no return.

Concluding remarks

In this review, we tried to illuminate the complexities of chromatin dynamics and illustrate how epigenetic changes impact adult stem cell fate determination and plasticity. Advances in genome technologies have provided means to not only comprehensively analyze the transcriptional profile of stem cells but also to map their epigenetic landscape on a global level and in their native niche. Although only in its infancy, several new concepts have emerged from recent studies: First, stem cell identity is governed by core transcription factor networks in conjunction with defined chromatin states. Once established, these states maintain robust lineage restriction and establish barriers against reversion to previous cellular states. During homeostasis, the niche microenvironment provides signals to reinforce cell fate decisions and maintains stem cells in an undifferentiated state. However, upon niche perturbations, stem cells can enter transitional states of plasticity until they reach their new microenvironment. This is achieved by coupling pioneer factors to chromatin platforms that are exquisitely sensitive to transcription factor concentrations, which in turn are modulated by microenvironmental cues.

Although the precise details of epigenetic networks in the regulation of adult stem cells remain to be clarified, modulation of epigenetic pathways should yield further potential for basic research and clinical applications alike (see Outstanding Questions).

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Glossary

Chromatin remodeling	dynamic modification of nucleosome structure, composition, and positioning to modulate gene expression
CpG island	short sequence of DNA with over-representation of CG dinucleotides compared to the genomic average. 40–70% of human promoters contain CpG islands
DNA methyltransferases (DNMTs)	Catalyze the methylation of DNA on cytosines in CpG dinucleotides. Methylation occurs on the fifth carbon of the pyrimidine ring of cytosines
Enhancer	regulatory DNA sequence containing multiple transcription factor binding sites. Enhancers activate transcription at a distance and independently of their orientation with respect to the target gene
Epicenter	short (<2kb) active subdomains within super-enhancers, which are particularly enriched for transcription factor binding sites and allow for cooperative binding. Epicenters are cell-stage specific and change dynamically depending on the microenvironment
Histone modifications	post-translational modifications whereby specific amino acid residues, particularly in histone tails, become chemically modified. These modifications include methylation, acetylation, phosphorylation, ubiquitination or ADP-ribosylation, and in a combinatorial manner, can affect histone–DNA interactions, histone–histone interactions, and the affinity for other proteins that regulate chromatin function. Specific histone modifications are linked to either an active or silenced chromatin state
Pioneer factors	a special class of transcription factors able to access their DNA target sites in silent chromatin. As such, pioneer factors establish competence for future gene expression by opening up local chromatin structure and facilitating the subsequent recruitment of additional transcription factors

Polycomb repressive complexes (PRC)	multi-protein complexes that reversibly modify chromatin structure and silence target genes
Stem cell niche	local tissue microenvironment that hosts and influences the behaviors or characteristics of stem cells
Stem cell plasticity	the ability of stem cells to adapt to a new microenvironment outside their niche and survive in limbo, e.g. in early stages of lineage progression, in wound-repair or in response to inflammation
Super-enhancer	Densely spaced clusters of active enhancers (>10kb) with unusually strong enrichment for the binding of cell-type specific transcription factors and transcriptional coactivators, including Mediator (MED1). Super-enhancers associate with critical cell-identity genes and thus dictate cellular behavior and fate

References

1. Bergman Y, Cedar H. DNA methylation dynamics in health and disease. *Nat. Struct. Mol. Biol.* 2013; 20:274–281. [PubMed: 23463312]
2. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. *Nat Rev Genet.* 2013; 14:204–220. [PubMed: 23400093]
3. Sharif J, et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature.* 2007; 450:908–912. [PubMed: 17994007]
4. Schübeler D. Function and information content of DNA methylation. *Nature.* 2015; 517:321–326. [PubMed: 25592537]
5. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci.* 2006; 31:89–97. [PubMed: 16403636]
6. Tahiliani M, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science.* 2009; 324:930–935. [PubMed: 19372391]
7. He YF, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. *Science.* 2011; 333:1303–1307. [PubMed: 21817016]
8. Ito S, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science.* 2011; 333:1300–1303. [PubMed: 21778364]
9. Zhang L, et al. Thymine DNA glycosylase specifically recognizes 5-carboxylcytosine-modified DNA. *Nat Chem Biol.* 2012; 8:328–330. [PubMed: 22327402]
10. Stadler MB, et al. DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature.* 2011; 480:490–495. [PubMed: 22170606]
11. Ziller MJ, et al. Charting a dynamic DNA methylation landscape of the human genome. *Nature.* 2013; 500:477–481. [PubMed: 23925113]
12. Khavari DA, et al. DNA methylation and epigenetic control of cellular differentiation. *Cell Cycle.* 2010; 9:3880–3883. [PubMed: 20890116]
13. Bock C, et al. DNA methylation dynamics during in vivo differentiation of blood and skin stem cells. *Mol. Cell.* 2012; 47:633–647. [PubMed: 22841485]
14. Hodges E, et al. Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment. *Mol. Cell.* 2011; 44:17–28. [PubMed: 21924933]
15. Tsumura A, et al. Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. *Genes Cells.* 2006; 11:805–814. [PubMed: 16824199]

16. Sen GL, et al. DNMT1 maintains progenitor function in self-renewing somatic tissue. *Nature*. 2010; 463:563–567. [PubMed: 20081831]
17. Li J, et al. Progressive alopecia reveals decreasing stem cell activation probability during aging of mice with epidermal deletion of DNA methyltransferase 1. *J Invest Dermatol*. 2012; 132:2681–2690. [PubMed: 22763785]
18. Pathania R, et al. DNMT1 is essential for mammary and cancer stem cell maintenance and tumorigenesis. *Nat. Commun*. 2015; 6:6910. [PubMed: 25908435]
19. Tsai CC, et al. Oct4 and Nanog directly regulate Dnmt1 to maintain self-renewal and undifferentiated state in mesenchymal stem cells. *Mol. Cell*. 2012; 47:169–182. [PubMed: 22795133]
20. Bröske AM, et al. DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. *Nat. Genet*. 2009; 41:1207–1215. [PubMed: 19801979]
21. Trowbridge JJ, et al. DNA methyltransferase 1 is essential for and uniquely regulates hematopoietic stem and progenitor cells. *Cell Stem Cell*. 2009; 5:442–449. [PubMed: 19796624]
22. Challen GA, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet*. 2011; 44:23–31. [PubMed: 22138693]
23. Challen GA, et al. Dnmt3a and Dnmt3b have overlapping and distinct functions in hematopoietic stem cells. *Cell Stem Cell*. 2014; 15:350–364. [PubMed: 25130491]
24. Wu H, et al. Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. *Science*. 2010; 329:444–448. [PubMed: 20651149]
25. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet*. 2012; 13:484–492. [PubMed: 22641018]
26. Maunakea AK, et al. Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Res*. 2013; 23:1256–1269. [PubMed: 23938295]
27. Maunakea AK, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature*. 2010; 466:253–257. [PubMed: 20613842]
28. Baubec T, et al. Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature*. 2015; 520:243–247. [PubMed: 25607372]
29. Zhang RR, et al. Tet1 regulates adult hippocampal neurogenesis and cognition. *Cell Stem Cell*. 2013; 13:237–245. [PubMed: 23770080]
30. Moran-Crusio K, et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell*. 2011; 20:11–24. [PubMed: 21723200]
31. Kunimoto H, et al. Tet2 disruption leads to enhanced self-renewal and altered differentiation of fetal liver hematopoietic stem cells. *Sci. Rep*. 2012; 2:273. [PubMed: 22355785]
32. Ko M, et al. Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. *Proc. Natl. Acad. Sci. U S A*. 2011; 108:14566–14571. [PubMed: 21873190]
33. Li Z, et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood*. 2011; 118:4509–4518. [PubMed: 21803851]
34. Quivoron C, et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer Cell*. 2011; 20:25–38. [PubMed: 21723201]
35. Mellén M, et al. MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system. *Cell*. 2012; 151:1417–1430. [PubMed: 23260135]
36. Spruijt CG, et al. Dynamic readers for 5-(hydroxy)methylcytosine and its oxidized derivatives. *Cell*. 2013; 152:1146–1159. [PubMed: 23434322]
37. Schwartz YB, Pirrotta V. A new world of Polycombs: unexpected partnerships and emerging functions. *Nat. Rev. Genet*. 2013; 14:853–864. [PubMed: 24217316]
38. Tavares L, et al. RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3. *Cell*. 2012; 148:664–678. [PubMed: 22325148]
39. Blackledge NP, et al. Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. *Cell*. 2014; 157:1445–1459. [PubMed: 24856970]

40. Lien WH, et al. Genome-wide maps of histone modifications unwind in vivo chromatin states of the hair follicle lineage. *Cell Stem Cell*. 2011; 9:219–232. [PubMed: 21885018]
41. Ezhkova E, et al. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell*. 2009; 136:1122–1135. [PubMed: 19303854]
42. Pereira J, et al. Ezh2, the histone methyltransferase of PRC2, regulates the balance between self-renewal and differentiation in the cerebral cortex. *Proc. Natl. Acad. Sci. U S A*. 2010; 107:15957–15962. [PubMed: 20798045]
43. Sen GL, et al. Control of differentiation in a self-renewing mammalian tissue by the histone demethylase JMJD3. *Genes Dev*. 2008; 22:1865–1870. [PubMed: 18628393]
44. Park DH, et al. Activation of neuronal gene expression by the JMJD3 demethylase is required for postnatal and adult brain neurogenesis. *Cell Rep*. 2014; 8:1290–1299. [PubMed: 25176653]
45. Shaw T, Martin P. Epigenetic reprogramming during wound healing: loss of polycomb-mediated silencing may enable upregulation of repair genes. *EMBO Rep*. 2009; 10:881–886. [PubMed: 19575012]
46. Shen X, et al. EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol. Cell*. 2008; 32:491–502. [PubMed: 19026780]
47. Ezhkova E, et al. EZH1 and EZH2 cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound repair. *Genes Dev*. 2011; 25:485–498. [PubMed: 21317239]
48. Frye M, Benitah SA. Chromatin regulators in mammalian epidermis. *Semin. Cell. Dev. Biol*. 2012; 23:897–905. [PubMed: 22944592]
49. Mochizuki-Kashio M, et al. Dependency on the polycomb gene Ezh2 distinguishes fetal from adult hematopoietic stem cells. *Blood*. 2011; 118:6553–6561. [PubMed: 22042701]
50. Hidalgo I, et al. Ezh1 is required for hematopoietic stem cell maintenance and prevents senescence-like cell cycle arrest. *Cell Stem Cell*. 2012; 11:649–662. [PubMed: 23122289]
51. Hwang WW, et al. Distinct and separable roles for EZH2 in neurogenic astroglia. *Elife*. 2014; 3:e02439. [PubMed: 24867641]
52. Juan AH, et al. Polycomb EZH2 controls self-renewal and safeguards the transcriptional identity of skeletal muscle stem cells. *Genes Dev*. 2011; 25:789–794. [PubMed: 21498568]
53. Chi AS, Bernstein BE. Developmental biology. Pluripotent chromatin state. *Science*. 2009; 323:220–221. [PubMed: 19131621]
54. Benoit YD, et al. Polycomb repressive complex 2 impedes intestinal cell terminal differentiation. *J. Cell Sci*. 2012; 125:3454–3463. [PubMed: 22467857]
55. Cantor AB, et al. Antagonism of FOG-1 and GATA factors in fate choice for the mast cell lineage. *J. Exp. Med*. 2008; 205:611–624. [PubMed: 18299398]
56. Pongubala JM, et al. Transcription factor EBF restricts alternative lineage options and promotes B cell fate commitment independently of Pax5. *Nat. Immunol*. 2008; 9:203–215. [PubMed: 18176567]
57. Schaffer AE, et al. Nkx6 Transcription Factors and Ptf1a Function as Antagonistic Lineage Determinants in Multipotent Pancreatic Progenitors. *Dev. Cell*. 2010; 18:1022–1029. [PubMed: 20627083]
58. Qi X, et al. Antagonistic regulation by the transcription factors C/EBP α and MITF specifies basophil and mast cell fates. *Immunity*. 2013; 39:97–110. [PubMed: 23871207]
59. Heinz S, et al. The selection and function of cell type-specific enhancers. *Nat Rev Mol Cell Biol*. 2015; 16:144–154. [PubMed: 25650801]
60. Hon GC, et al. 5mC oxidation by Tet2 modulates enhancer activity and timing of transcriptome reprogramming during differentiation. *Mol. Cell*. 2014; 56:286–297. [PubMed: 25263596]
61. Creighton MP, et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl. Acad. Sci. U S A*. 2010; 107:21931–21936. [PubMed: 21106759]
62. Ernst J, et al. Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature*. 2011; 473:43–49. [PubMed: 21441907]
63. Heintzman ND, et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature*. 2009; 459:108–112. [PubMed: 19295514]

64. Rada-Iglesias A, et al. A unique chromatin signature uncovers early developmental enhancers in humans. *Nature*. 2011; 470:279–283. [PubMed: 21160473]
65. Zaret KS, Carroll JS. Pioneer transcription factors: establishing competence for gene expression. *Genes Dev*. 2011; 25:2227–2241. [PubMed: 22056668]
66. Soufi A, et al. Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. *Cell*. 2012; 151:994–1004. [PubMed: 23159369]
67. Soufi A, et al. Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming. *Cell*. 2015; 161:555–568. [PubMed: 25892221]
68. Heinz S, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell*. 2010; 38:576–589. [PubMed: 20513432]
69. Meredith DM, et al. Program specificity for Ptf1a in pancreas versus neural tube development correlates with distinct collaborating cofactors and chromatin accessibility. *Mol. Cell. Biol*. 2013; 33:3166–3179. [PubMed: 23754747]
70. Gertz J, et al. Distinct properties of cell-type-specific and shared transcription factor binding sites. *Mol. Cell*. 2013; 52:25–36. [PubMed: 24076218]
71. Pimkin M, et al. Divergent functions of hematopoietic transcription factors in lineage priming and differentiation during erythro-megakaryopoiesis. *Genome Res*. 2014; 24:1932–1944. [PubMed: 25319996]
72. Junion G, et al. A transcription factor collective defines cardiac cell fate and reflects lineage history. *Cell*. 2012; 148:473–486. [PubMed: 22304916]
73. Yan J, et al. Transcription factor binding in human cells occurs in dense clusters formed around cohesin anchor sites. *Cell*. 2013; 154:801–813. [PubMed: 23953112]
74. Wilson NK, et al. Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. *Cell Stem Cell*. 2010; 7:532–544. [PubMed: 20887958]
75. Liu Z, et al. Enhancer activation requires trans-recruitment of a mega transcription factor complex. *Cell*. 2014; 159:358–373. [PubMed: 25303530]
76. Rada-Iglesias A, et al. Epigenomic annotation of enhancers predicts transcriptional regulators of human neural crest. *Cell Stem Cell*. 2012; 11:633–648. [PubMed: 22981823]
77. Wamstad JA, et al. Dynamic and coordinated epigenetic regulation of developmental transitions in the cardiac lineage. *Cell*. 2012; 151:206–220. [PubMed: 22981692]
78. Whyte WA, et al. Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell*. 2013; 153:307–319. [PubMed: 23582322]
79. Lovén J, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell*. 2013; 153:320–334. [PubMed: 23582323]
80. Hnisz D, et al. Super-enhancers in the control of cell identity and disease. *Cell*. 2013; 155:934–947. [PubMed: 24119843]
81. Adam RC, et al. Pioneer factors govern super-enhancer dynamics in stem cell plasticity and lineage choice. *Nature*. 2015; 521:366–270. [PubMed: 25799994]
82. Rhee H, et al. Lhx2 maintains stem cell character in hair follicles. *Science*. 2006; 312:1946–1949. [PubMed: 16809539]
83. Vidal VP, et al. Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. *Curr. Biol*. 2005; 15:1340–1351. [PubMed: 16085486]
84. Nowak JA, et al. Hair follicle stem cells are specified and function in early skin morphogenesis. *Cell Stem Cell*. 2008; 3:33–43. [PubMed: 18593557]
85. Horsley V, et al. NFATc1 balances quiescence and proliferation of skin stem cells. *Cell*. 2008; 132:299–310. [PubMed: 18243104]
86. Nguyen H, et al. Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia. *Nat. Genet*. 2009; 41:1068–1075. [PubMed: 19718027]
87. Folgueras AR, et al. Architectural niche organization by LHX2 is linked to hair follicle stem cell function. *Cell Stem Cell*. 2013; 13:314–327. [PubMed: 24012369]

88. Keyes BE, et al. Nfatc1 orchestrates aging in hair follicle stem cells. *Proc. Natl. Acad. Sci. U S A*. 2013; 110:E4950–4959. [PubMed: 24282298]
89. Kadaja M, et al. SOX9: a stem cell transcriptional regulator of secreted niche signaling factors. *Genes Dev*. 2014; 28:328–341. [PubMed: 24532713]
90. Lien WH, et al. In vivo transcriptional governance of hair follicle stem cells by canonical Wnt regulators. *Nat. Cell Biol*. 2014; 16:179–190. [PubMed: 24463605]
91. Gosselin D, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell*. 2014; 159:1327–1340. [PubMed: 25480297]
92. Lavin Y, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. *Cell*. 2014; 159:1312–1326. [PubMed: 25480296]
93. Rompolas P, et al. Spatial organization within a niche as a determinant of stem-cell fate. *Nature*. 2013; 502:513–518. [PubMed: 24097351]
94. van Es JH, et al. Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat. Cell Biol*. 2012; 14:1099–1104. [PubMed: 23000963]
95. Tian H, et al. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature*. 2011; 478:255–259. [PubMed: 21927002]
96. Yan KS, et al. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. *Proc. Natl. Acad. Sci. U S A*. 2012; 109:466–471. [PubMed: 22190486]
97. Buczacki SJ, et al. Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature*. 2013; 495:65–69. [PubMed: 23446353]
98. Rompolas P, et al. Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. *Nature*. 2012; 487:496–499. [PubMed: 22763436]
99. Spradling A, et al. Stem cells find their niche. *Nature*. 2001; 414:98–104. [PubMed: 11689954]
100. Scadden DT. The stem-cell niche as an entity of action. *Nature*. 2006; 441:1075–1079. [PubMed: 16810242]
101. Hsu YC, et al. Dynamics between stem cells, niche, and progeny in the hair follicle. *Cell*. 2011; 144:92–105. [PubMed: 21215372]
102. Hsu YC, et al. Transit-amplifying cells orchestrate stem cell activity and tissue regeneration. *Cell*. 2014; 157:935–949. [PubMed: 24813615]
103. Kim TH, et al. Broadly permissive intestinal chromatin underlies lateral inhibition and cell plasticity. *Nature*. 2014; 506:511–515. [PubMed: 24413398]
104. Luyten A, et al. Active enhancers are delineated de novo during hematopoiesis, with limited lineage fidelity among specified primary blood cells. *Genes Dev*. 2014; 28:1827–1839. [PubMed: 25128499]
105. Lara-Astiaso D, et al. Immunogenetics. Chromatin state dynamics during blood formation. *Science*. 2014; 345:943–949. [PubMed: 25103404]
106. Hnisz D, et al. Convergence of developmental and oncogenic signaling pathways at transcriptional super-enhancers. *Mol. Cell*. 2015; 58:362–370. [PubMed: 25801169]
107. Blanpain C, Fuchs E. Stem cell plasticity. Plasticity of epithelial stem cells in tissue regeneration. *Science*. 2014; 344:1242281. [PubMed: 24926024]
108. Ito M, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat. Med*. 2005; 11:1351–1354. [PubMed: 16288281]
109. Banerji J, et al. Expression of a β -globin gene is enhanced by remote SV40 DNA sequences. *Cell*. 1981; 27:299–308. [PubMed: 6277502]

Trends

- Adult stem cells coordinate niche signals and chromatin states to choose appropriate fates. Upon changes in the local niche environment, stem cells remodel chromatin to survive in transitional states, before undergoing fate selection.
- Epigenetic repressors put a brake on precocious lineage commitment. DNA methylation and Polycomb-silencing complexes cooperate to ensure that stem cells are robustly maintained during homeostasis.
- Cell identity depends on combinatorial transcription factor complexes on lineage-specific enhancers. Pioneer factors select unique enhancer repertoires by making condensed chromatin accessible for robust gene activation.
- Epigenetic memory is achieved by coupling pioneer factors with super-enhancers, allowing stem cells to retain their unique identities in different microenvironments.

BOX 1**Cellular plasticity – stem cells remain true to themselves**

Adult stem cells are characterized by their ability to self-renew for long-term and to produce differentiated cell lineages. As such, they are used sparingly and their main function is to fuel tissue homeostasis. However, it has become increasingly clear that the fate and multi-lineage potential of adult stem cells can change depending on whether a stem cell exists within its resident niche, whether it is mobilized to repair a wound, or whether it is challenged to *de novo* tissue morphogenesis after expansion in culture and following transplantation [107]. As such, cellular plasticity is the ability of stem cells to adapt to a new microenvironment outside the niche and survive in limbo. Therefore, plasticity is not tested until cells are faced with a new microenvironment.

In most mammals, the epidermis has a dense array of hair follicles, which typically make negligible contribution to epidermal homeostasis. Upon injury, however, hair follicle stem cells efficiently migrate out of their niche and into the epidermis, where they contribute long-term to wound repair. In the process, these stem cells lose hair follicle markers and adopt features of epidermal stem cells [108]. Plasticity is not a feature that is limited to stem cells. After ablation of mammalian epithelial stem cells, either by laser or using diphtheria toxin, the empty niche can recruit and induce normally committed cells to proliferate and revert back to a progenitor-like state. Indeed, hair follicle stem cells can be replaced by committed cells above the niche, while hair germ cells can be readily replenished if hair follicle stem cells are intact [93,98]. Similarly in the intestinal crypt, loss of LGR5+ stem cells triggers dedifferentiation of committed precursor cells into functional stem cells, which then repopulate the crypt [94,97]. Collectively, these studies have uncovered the dramatic plasticity within mammalian tissues following injury. Stem cells can acquire greater fate flexibility to replenish multiple lineages, whereas upon stem cell loss, their progeny and even differentiated cells may dedifferentiate to repair tissue damage.

BOX 2**Super-enhancers in the control of stem cell identity**

Initial studies using viral SV40 DNA in the 1980s revealed that sequences remote from gene promoters can readily boost transcription levels [109]. These genetic elements were appropriately termed ‘enhancers’, which are functionally defined as short DNA sequences with the potential to increase basal transcription levels from a distance and independently of orientation on DNA. Mechanistically, enhancers contain binding sites for transcription factors, which cooperate with Mediator to recruit RNA Polymerase and initiate gene transcription [59]. Recent studies on cultured embryonic stem cells have shown that besides the short typical enhancers, a special set of large open-chromatin domains, so called ‘super-enhancers’, control the expression of genes particularly important in stem cell behavior [78]. These super-enhancers differ from typical enhancers by their exceptional size (>10kb) and their particular enrichment for factors generally associated with enhancer activity. These include a high density of H3K27ac and H3K4me1 modifications, increased Mediator subunit MED1 and RNA polymerase II occupancy, enrichment of RNA from transcribed enhancers (eRNA), elevated binding of histone acetyltransferases p300/CBP, as well as generally increased chromatin accessibility as measured by DNase-seq [80]. Importantly, super-enhancers are also characterized by their high density of sequence motifs for cell stage-specific transcription factors. This allows for their cooperative binding, thereby rendering super-enhancer regulated genes particularly sensitive to the key transcription factor cohort. Notably, the genes encoding lineage-specific transcription factors often themselves harbor super-enhancers, resulting in a stable feed-forward loop to fuel and maintain the lineage [78,81].

Studies in adult stem cells have recently uncovered a hitherto unappreciated complexity in organization, constituency and dynamics of super-enhancers. Within super-enhancers, smaller segments or ‘epicenters’ exist where the concentration of binding sites for stemness transcription factors is particularly high (Figure 1A) [81]. As such, epicenters are cell-stage specific and serve as highly accurate chromatin sensors that respond to local changes in the microenvironment. Moreover, epicenters cloned from super-enhancers were sufficient to target proper developmental-specific behavior of an eGFP reporter (Figure 1B) [81], underscoring the functional relevance of these chromatin domains in lineage-specific gene expression. Thus, epicenters harbor all the information necessary to express genes in the right place and time during development. This discovery should yield powerful genetic tools to drive expression with unprecedented precision.

Outstanding questions

- What are the relative contributions of different epigenetic modifications, and how are these pathways integrated at the molecular level to prompt stem cells to embark on a specific lineage?

Recent evidence has shown that no single pathway acts dominantly in the control of lineage choice. Instead, the emerging picture suggests that stem cells intricately rely on the concerted action of multiple epigenetic pathways, which act synergistically to govern fate decisions.

- How do external signals interface with enhancers to elicit chromatin changes and fate commitment *in vivo*?

Although signaling pathways (e.g. WNTs, BMPs) influence the biology of nearly all adult stem cells, their impact on chromatin remodeling is still poorly understood. Chromatin profiling of early stem cell progeny, where these pathways are dynamically changed, should yield detailed insights into the temporal progression of lineage commitment.

- What is the hierarchical organization of the transcriptional network that specifies distinct cellular fates?

There is substantial evidence that pioneer factors bind nucleosomal DNA and initiate the cascade of remodeling events to initiate gene expression. However, the temporal order in which nucleosomes are repositioned, DNA is demethylated, histones are modified and additional transcription factors bind is not well defined and awaits further characterization.

- What is the molecular basis for the dose-dependent effects of pioneer factors on the stem cell regulatory program?

The notion that pioneer factors can recruit multi-protein complexes implies that their expression needs to be in equilibrium with other cofactors to maintain cellular state. Changing the levels of pioneer factors may allow cells to alter the composition of transcription factor complexes and direct them to DNA binding sites with different affinities. This could ultimately allow cells to adapt and retain stemness in the face of environmentally-induced transcription factor landscape changes.

Figure 1. Dynamic chromatin remodeling during stem cell lineage commitment

(A) In adult stem cells, differentiation-associated gene promoters are frequently silenced by the concerted action of Polycomb-repressive complexes (PRC) and DNA methyltransferases (DNMT3A/B). The PRC2 component EZH2 deposits the silencing mark H3K27me3, which can be recognized by the PRC1 moiety CBX. This facilitates PRC1 recruitment and allows for RING1A/B mediated deposition of H2AK119ub, which further leads to chromatin compaction. DNMT3A/B can methylate CpG islands at promoters and contribute to gene silencing, while histone deacetylases (HDACs) remove active chromatin marks (e.g. H3K27ac). This ultimately results in condensed chromatin and potent repression of gene expression.

(B) Upon stem cell lineage commitment, dynamic chromatin remodeling is required to induce a program of differentiation. TET enzymes remove 5mCpG through iterative oxidation, whereas histone demethylases (e.g. JMJD3) eliminate repressive chromatin marks (e.g. H3K27me3). On the other hand, histone methyltransferases (HMTs) establish a permissive chromatin environment, for example by deposition of the H3K4me1 (at enhancers) or H3K4me3 (at promoters) marks. Upon external niche stimuli, pioneer factors engage their target sites in silent chromatin, and recruit histone acetyl transferases (p300/CBP, which deposit H3K27ac) as well as other transcription factors. Although the temporal order of chromatin remodeling is unclear, transcription factors at enhancers can eventually be recognized by the Mediator co-activator complex, which recruits RNA Polymerase II to gene promoters, resulting in de-repression of gene activity and the induction of stem cell differentiation.

Figure I. Super-enhancer epicenters confer tissue-, lineage- and temporal-specificity. (within Box 2)

(A) Hair follicle stem cell super-enhancers consist of clusters of active enhancers with exceptionally high density of H3K27ac (ChIP-seq) and transcriptional activators (adapted from [81]). Red box highlights one of the hair follicle stem cell super-enhancer epicenters. The schematic illustrates that epicenters are short (<2kb) DNA segments, densely bound by multiple cell-stage specific transcription factors and co-activators. In hair follicle stem cells, SOX9 acts as pioneer factor, which recruits other transcription factors, Mediator (MED1) and histone acetyltransferases (p300, CBP). Active enhancers are also depleted of 5mC (through TET enzymes) and display high levels of H3K4me1 (through histone methyltransferases, HMTs).

(B) Co-occupancy (ChIP-seq) of hair follicle stem cell transcription factors and Mediator subunit MED1 occurs within super-enhancer epicenters (left, adapted from [81]). To test whether epicenters faithfully drive reporter gene expression *in vivo* (green fluorescent protein, eGFP, coupled with a minimal SV40 promoter under the control of the epicenter), high-titer lentivirus (LV, middle) was injected into the amniotic cavity of E9.5 mouse embryos. This results in stable integration of the reporter construct into skin progenitor chromatin and propagation into adult mice. The immunofluorescence image (right) marks the nuclei of the skin in blue (DAPI), the hair follicle stem cells in red (keratin 24), and a subset of stem cells that received the viral reporter. Note absence of eGFP in other skin cells (see also [81]). These findings illustrate that epicenters of quiescent hair follicle stem cells are only active in these cells, highlighting the cell-stage specificity of these special open chromatin domains.