Deciphering the Epigenetic Code in Embryonic and Dental Pulp Stem Cells

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A close cooperation between chromatin states, transcriptional modulation, and epigenetic modifications is required for establishing appropriate regulatory circuits underlying self-renewal and differentiation of adult and embryonic stem cells. A growing body of research has established that the epigenome topology provides a structural framework for engaging genes in the non-random chromosomal interactions to orchestrate complex processes such as cell–matrix interactions, cell adhesion and cell migration during lineage commitment. Over the past few years, the functional dissection of the epigenetic landscape has become increasingly important for understanding gene expression dynamics in stem cells naturally found in most tissues. Adult stem cells of the human dental pulp hold great promise for tissue engineering, particularly in the skeletal and tooth regenerative medicine. It is therefore likely that progress towards pulp regeneration will have a substantial impact on the clinical research. This review summarizes the current state of knowledge regarding epigenetic cues that have evolved to regulate the pluripotent differentiation potential of embryonic stem cells and the lineage determination of developing dental pulp progenitors.

INTRODUCTION

In recent years, the field of epigenetics has grown into one of the most rapidly expanding research endeavors to date [1-4]. An increasing amount of evidence suggests that tooth development, enamel formation, and periodontal disease require tight coordination between transcriptional regulation and epigenetic modifications in stem cell precursors [5-12]. Among stem cells, dental pulp stem cells (DPSCs†) represent a unique population of precursor cells isolated from the postnatal human dental pulp capable of regenerating a reparative dentin-like complex [13,14]. DPSCs retain the ability to differentiate into different cell types including melanocytes, odonoblasts, osteoblasts, chondrocytes, adipocytes, neural progenitors, cells of blood vessels, and smooth muscle [15-18]. The following characteristics such as relative abundance, low morbidity, differentiation potential and tolerance to biomaterials make DPSCs highly promising for tooth tissue engineering and craniomaxillofacial regeneration [19-24]. Moreover, the capacity of DPSCs to differentiate into specialized cells makes them attractive candidates for clinical applications [25-31].

DPSCs originate from the cranial neural crest (NC) and have some characteristics of neural crest progenitors, a migratory cell population that gives rise to a broad range of cell types [32-35]. It has been proposed that evolution of the NC-specification program has enabled cells at the neural plate border to acquire multipotency and migratory ability [36]. A subgroup of transcription factors termed neural plate border specifiers is required for the establishment of the neural plate border (Figure 1), as well as regulation of the downstream target factors known as neural crest specifiers, which mediate the induction of neural crest lineage [35].

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†Abbreviations: CTCF, CCCTC-binding zinc finger transcription factor; CpG, cytosine and guanine rich dinucleotides; CGIs, CpG islands; DNMT, DNA methyltransferases; DF, dental follicle; DP, dental pulp; DPSC, dental pulp stem cells; eRNA, enhancer RNA; ESC, embryonic stem cells; FRα, folate receptor alpha; HAT, histone acetyltransferase; HDACs, histone deacetyltransferases; HDACi, histone deacetylase inhibitors; HMT, histone methyltransferase; LPS, bacterial lipopolysaccharide; lncRNA, long non-coding RNA; MAPK, mitogen-activated protein kinase; MBD, methyl CpG-binding protein; miRs, microRNA; NC, neural crest; NuRD, nucleosome remodeling deacetylase; Pol II, RNA Polymerase II; PGI, polycomb; PRC, polycomb repressive complex; TAD, topologically associating domain; TGG, thymine DNA glucosylase; TET, ten-eleven translocation protein; 3D, the three-dimensional; TrxG, trithorax; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; 5fC, 5-formylcytocine; 5 caC, 5-carboxycytocine; 5-aza-Cdr, 5-aza-2-deoxycytidine.

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There is growing evidence that the epigenetic landscape underlies the dynamic behavior of regulatory circuits orchestrating the NC fate specification [35,37-39]. In this regard, DNA methyltransferase DNMT3A has been shown to be critical for enabling the activation of NC specifier Gbx2 (Figure 1), an essential transcription factor for normal development of the inner ear [40]. In another study, DNA demethylation, which is mediated by the TeT-TDG enzymatic complex, has been shown to regulate the formation of NC structures [41]. The NeIL family of DNA glycosylases cooperates with thymine DNA glycosylase (TDG), TET dioxygenases, and DNA methyltransferases (DNMT) to control target genes. The expression of pluripotency genes including Oct4, Sox2, and Klf4 can be regulated by microRNAs. FRα, folic acid receptor, can activate pluripotency genes via microRNA inhibition.

Figure 1. The self-renewal potential of embryonic stem cells (ESC), neural crest stem cells (NCSC) and dental pulp stem cells (DPSC) is dependent upon a shared gene regulatory network of self-renewal factors (left) and neural crest specifiers (right). The combinatorial activity of different epigenetic enzymes and chromatin remodeling complexes ultimately fine-turns the transcriptional output of cognate genes in neural crest in response to diverse signaling (bottom). FoxD3 binds to SWI/SNF complex to facilitate transcriptional activation, while interaction between FoxD3 and histone deacetylases (HDACs) attenuates the expression of occupied genes. The NEIL family of DNA glycosylases cooperates with thymine DNA glycosylase (TDG), TET dioxygenases, and DNA methyltransferases (DNMT) to control target genes. The expression of pluripotency genes including Oct4, Sox2, and Klf4 can be regulated by microRNAs. FRα, folic acid receptor, can activate pluripotency genes via microRNA inhibition.
For instance, the Polycomb complex, well known for its genome-wide epigenetic repression, is involved in the structural reorganization of the 3D genome during stem cell differentiation as well as the maintenance of cellular memory [59,60].

The origin of dental pulp suggests that DPSCs have inherited a subset of NC-specific modules from the predecessor regulatory network (Figure 1). The combinatorial activity of some NC specifiers is most likely to contribute to the formation of the dental pulp-specific regulatory nodes that carry the molecular blueprint of orofacial and dental development. The common origin of osteoblasts, cementoblasts, and odontoblasts from the cranial NC is reflected in the similarity of gene expression profiles, although distinct epigenomic states delineate transcriptional programs during cell fate determination [61,62]. The regulatory genes encoding the pluripotency transcription factors and the NC specifiers including Oct4, Nanog, Rex1, Sox2 and FoxD3 have been identified in mouse DPSCs [63]. The relationship between these master regulators is complex and has been linked to self-renewal and differentiation. There is evidence that OCT4, NANOG, SOX2 and STAT3 contribute significantly to terminal differentiation of ameloblasts and odontoblasts in the tooth germ of human fetuses [64]. It was also documented that OCT4/NANOG axis maintains the mesenchymal stem cell-like property in the human DPSCs [65]. Upon differentiation of ESCs to epiblast stem cells, the forkhead transcription factor FoxD3 facilitates the simultaneous establishment of active and repressive chromatin configuration at gene targets [66]. FoxD3 is able to interact with histone deacetylases to attenuate activation of its cognate enhancers, while concurrently recruiting the SWI/SNF chromatin-remodeling complex to promote a more open chromatin via nucleosome eviction at the bound sites (Figure 1). Recently, Fujita et al. observed that during specification of mouse NC stem cells, master regulators Oct3/4, Sox2, and Nanog as well as chromatin remodeling factor CHD7 co-bind at the H3K4me1/me3-positive regulatory elements of FoxD3 [67].

**EPIGENETIC MECHANISMS IN EMBRYONIC STEM CELLS**

Although this overview highlights the latest advances in the understanding of the epigenetic processes underlying the molecular control of DPSCs, I will first summarize the current state of knowledge regarding the regulatory framework, which orchestrates ESC specification, commitment and differentiation. A more detailed discussion of the epigenetic events underlying stem cell differentiation, cellular reprogramming and development can be found in a number of excellent review articles that have been published within the last few years [68-70].

Based on their shared molecular pathways, epigenetic modulators can be divided into three functional categories [71]. Epigenetic “writer” is any protein or protein module that catalyzes the transfer of chemical groups onto N-terminal histone tails; epigenetic “eraser” is any protein or protein module that is capable to remove a chemical group and epigenetic “reader” is any protein or protein module that can decipher a specific chemical group for transmitting information into the structural changes within a discrete chromatin domain (Figure 2).

**Histone Acetylation**

Acetylation of the lysine residues at the N terminus of histones removes positive charges, thereby exacerbating the affinity between histones and DNA creating a more relaxed chromatin conformation permissible for transcription [72].

The genomic region surrounding transcriptionally active genes is enriched in H3K4ac, H3K27ac and H3K39ac [73-76]. Histone acetylation on H3K27 distinguishes active enhancers (Figure 3), while poised enhancers associate with H3K4me1 [73,74]. The histone acetylation...
signature at H3K9, H4K12, H3K14, H3K27, and H3K122 defines active enhancers that are linked to CpG-rich islands and bivalent chromatin [77-79]. Interestingly, in the four-cell stage human embryos, H4K12ac is deposited in the vicinity of genes that control histone folding and DNA-dependent transcription, while in human blastocysts, the same histone mark is enriched at key developmental genes [78]. Although H3K27ac is a hallmark feature of active enhancers, a subset of active enhancers is marked with H4K16ac, H3K56ac and H2BK20ac [80-83]. Moreover, Pradeepa et al. have shown that H3K64ac and H3K122ac extend over active promoters [84]. Interestingly, this study has also revealed a novel subclass of active enhancers that lack H3K27ac, but positive for H3K122ac. In another report, hyperacetylation at H3K4, 9, 14, 18, 56 and 122 as well as acetylation at H4K5, 8, 12 and 16 (Figure 3) has been linked to the pluripotency state of human ESCs [85].

Histone acetyltransferases (HATs) mediate the transfer of an acetyl group to histones, transcription factors and other chromatin-associated proteins [86,87]. CREB-binding protein CBP and e1A-binding protein p300, two highly conserved HATs, transform condensed chromatin into a more relaxed structure by acetylating histones, transcription factors and coactivator complexes associated with the basal transcription machinery [88]. The Wnt signaling pathway was shown to shift the balance between cell proliferation and cell fate specification through the CBP/β-catenin or p300/β-catenin-dependent mechanisms [89]. H3K56ac, a substrate of CBP/p300 (Table 1), marks chromatin domains with high nucleosome turnover in nuclear processes that are linked to gene transcription and DNA replication [82,83]. Rapid changes in H3K56 acetylation at the Notch-regulated enhancers require CBP binding, and reliably reflect enhancer activation [90]. In human ESCs, the recruitment of NANOG, SOX2, and OCT4 to the target promoters is associated with chromatin domains enriched in H3K56ac [91]. In addition to histone acetylation at H3K56, CBP and p300 contribute to a genomewide acetylation on H3K18 and H3K27 [92].

The MYST2 histone acetyltransferase ensures naïve pluripotency and ESC self-renewal and has also been shown to be indispensable for early mouse post-implantation development [93,94]. Histone acetylation catalyzed by MYST2 is an intermediate step for the recruitment of Oct4 to the Nanog promoter indicating that MYST is an essential chromatin-remodeling enzyme required for the maintenance of pluripotency [93]. The activity of MOF is
another MYST family member, which catalyzes the transfer of acetyl groups to H4K16 and is enriched at bivalent chromatin [95,96]. Depletion of mouse Mo is affects the expression of Nanog, Oct4, and Sox2 changing the potential of the pluripotent end-state [95]. MOZ, a member of the MYST family, and the Polycomb-family repressor BMI1, participate in the repression of Hox genes in differentiating ESCs [97]. The double PHD finger of MYST family was implicated in promoting H3K9ac and H3K14ac histone marks [98]. GCN5, a component of the SAGA and ATAC lysine acetyltransferase complexes, is important for acetylation on H4K12 and H4K16 during the stem cell reprogramming [99]. GCN5 associates with Myc to trigger the activation of a distinct alternative splicing network leading to the early acquisition of pluripotency-associated splicing state [100].

Although it is a common belief that histone acetylation is a prerequisite signature of gene activation, new data links histone acetylation to gene silencing. It has recently been documented that H4K20ac sponsors gene repression in HeLa-S3 cells [101].

### Table 1. Epigenetic modifications in embryonic stem cells

| Family                  | Substrate specificity | Molecular function                  | References  |
|-------------------------|-----------------------|-------------------------------------|-------------|
| **Histone acetyltransferases** |                       |                                     |             |
| p300/CBP                | H3K27, H3K18, H3K56   | active enhancers                    | [82,83,90-92]|
| MYST/MOZ                | H4K16, H3K9, H3K14    | active enhancers                    | [96,98]     |
| GCN5                    | H4K12, H4K16          | gene activation                      | [99]        |
| BRD4                    | H3K122                | nucleosome eviction                  | [184]       |
| **Histone deacetylases** |                       |                                     |             |
| HDAC1/2                 | H3K56ac, H3K27ac, H3K9ac | NuRD/CoREST repressor             | [104,112]   |
| Sirtuins (SIRT1, SIPT6) | H4K16ac, H3K9ac       | subunit of PRC4 repressor           | [113,120]   |
| **Histone methyltransferases** |                   |                                     |             |
| EZH2                    | H3K27me3              | subunit of PRC2                     | [121-124]   |
| SUV3/9H1                | H3K9me2/me3           | repressed chromatin                  | [134,135]   |
| G9A/GLP                 | H3K9me2, H3K27me3     | repression                           | [136,139,140]|
| SETD2/7                 | H3K36me3              | repression                           | [142-144]   |
| MLL1-4                  | H3K4me3, H3K4me1      | SET/COMPASS activator               | [124,147]   |
| **Histone demethylases** |                       |                                     |             |
| LSD1/2                  | H3K4me2, H3K27me3     | CoREST repressor                    | [157,158]   |
| KDM2B                   | H3K36me3              | recruits RING1B/PRC1                 | [130-132]   |
| KDM4B/C                 | H3K4me3               | block H3K9m3/K36me3                 | [163]       |
| KDM5B                   | H3K4me3               | binds to PRC2 and LSD1              | [165,166]   |
| KDM6A/B                 | H3K27me3              | β-catenin recruitment               | [168-170]   |
| PHF8                    | H3K9me2               | removes repressive marks            | [171]       |
| **Monoubiquitin ligases** |                       |                                     |             |
| RING1B                  | H2AK119ub1            | recruitment of PRC2                 | [128,174]   |
| RNF20                   | H2BK120ub1            | lineage commitment                   | [177]       |
| **DNA methyltransferases** |                     |                                     |             |
| DNMT1, DNMT3A, DNMT3B   | DNA methylation       | gene repression                      | [69]        |
| **DNA hydroxylases**    |                       |                                     |             |
| TET1-3                  | convert 5mC to 5hmC, 5fC and 5caC | activation/repression               | [200-211]   |
transcriptional activators are excluded from the H4K20ac-enriched loci.

**Histone Deacetylation**

Histone deacetylases (HDACs) play a crucial role in the maintenance and differentiation of stem cells by mediating chromatin condensation and transcriptional silencing [102]. HDACs represent a class of enzymes that removes acetyl groups from the N-terminal histone tails [103]. HDAC1 and HDAC2 participate in chromatin organization and gene regulation as the core catalytic components of Sin3A, NuRD, and CoREST co-repressor complexes [102,103]. The inactivation of HDAC1, but not HDAC2, can cause elevated H3K56ac levels [104]. During early ESC differentiation, HDAC1 removes acetyl groups from key pluripotency genes [105]. Ablation of HDAC1 leads to an increase in H3K9ac and loss of DNA methylation [106].

Although the primary function of HDACs is gene silencing, several studies have linked HDACs to transcriptional activation of the pluripotent and self-renewal genes [107]. An immediate reduction in cell viability and decreased expression of Oct4, Nanog, Esrrb, and Rex1 is a result of HDAC1 and HDAC2 depletion [108].

Sin3/NuRD is a corepressor complex, which is implicated in the regulation of essential biological processes including cellular proliferation, differentiation, apoptosis, and cell cycle [109]. The Nucleosome Remodeling and Deacetylase (NuRD) is another complex composed of multiple subunits, including HDAC1/2, the ATP-dependent remodeling enzymes CHD3/4, histone chaperones RbAp46/48, the CpG-binding proteins MBD2/3, p66α/β and specific DNA-binding proteins of the MTA family [110]. The balance between acetylation and methylation of histones controlled by NuRD is required for fine-tuning transcription of developmental genes as well as maintaining the differentiation response of pluripotent stem cells [111,112]. Deacetylation of H3K27ac by NuRD facilitates the recruitment of Polycomb and subsequent deposition of a repressive H3K27me3 mark at the NuRD-bound promoters [112].

Sirtuins belong to a large family of deacetylases that play a major role in maintaining genome integrity, largely through targeting different histone marks, including H4K16ac, H3K9ac, H3K56ac, and H3K18ac [113]. SIRT1, a subunit of the Polycomb repressive complex PRC4, is involved in the acquisition of stress resistance, metabolism, hematopoiesis, aging, and tumor suppression [114,115]. In response to endogenous oxygen, SIRT1 blocks nuclear translocation of cytoplasmic p53 and triggers mitochondrial-dependent apoptosis in mouse ESCs [116]. SIRT1 is able to influence early cell fate decisions via the p53-mediated regulation of the Nanog gene [117]. In addition, SIRT1 facilitates the PTEN/JNK/FOXO1 signaling response to reactive oxygen species and regulates the induction of autophagy and mitochondrial function in ESCs under oxidative stress [118,119]. SIRT6, another Sirtein family member, safeguards the balance between pluripotency and differentiation through the TET-mediated production of 5-hydroxymethylcytosine (5hmC) [120]. Depletion of SIRT6 leads to augmented levels of 5hmC in the neural-specific epigenomic environment causing derepression of mouse Oct4, Sox2, and Nanog genes.

**Histone Methylation**

Histone methylation is mediated by histone methyltransferases (HMT) that catalyze the transfer of one, two, or three methyl groups to lysine and arginine residues of histone proteins. A regulated crosstalk between closed and open chromatin is mediated by the interplay of Polycomb group (PcG) and Trithorax group (TrxG) complexes [121-124]. PcG is part of the multi-subunit Polycomb Repressive Complexes PRC1 and PRC2, which are responsible for histone methylation and chromatin compaction. PRC2 contains four functional subunits EZH1/2, SUZ12, EED and RbBP4 (Figure 4A). EZH2, an essential component of PRC2, catalyzes the transfer of methyl groups on H3K27 [121-124]. In ESCs, developmental genes are poised for expression because their promoters are marked with H3K27me3/K4me3 bivalent chromatin [125]. The proportion of tissue-specific genes in a repressed state was shown to be significant, despite the presence of H3K4me3 at their promoters. It has been proposed that bivalent chromatin represents a major landmark to distinguish different cell types, rather than poising gene expression during differentiation [125].

PRC1 is composed of six major parts, each containing a distinct PCGF subunit, H2A monoubiquitin ligases RING1A/B and a unique set of PRC1-associated proteins [126]. PRC1 and PRC2 catalyze mono-ubiquitination on histone H2A (H2AK119ub1), which serves as a nucleation site for Polycomb to promote the subsequent deposition of H3K27me3 [127-129]. Mechanistically, PRC1 initiates ubiquitin transfer to H2AK119 (Figure 4B) leading to recruitment of PRC2 and H3K27me3 deposition [130]. Histone demethylase KDM2B interacts with RING1B to facilitate the recruitment of PRC1 to its target genes, thereby promoting H2AK119 ubiquitination in ESCs [131,132].

SUV39H1, another critical HMT, deposits H3K9me3 and HP1α to the promoter of Oct4 in differentiating mouse ESCs [133]. To fulfill its repressive function, SUV39H1 forms a regulatory complex with a specific long non-coding RNA transcribed from the pseudogene Oct4P4. The genomic regions co-occupied by SUV39H1 and IncRNA are also enriched in chromatin factors DAXX and ATRX, which safeguard the genome by silencing repetitive elements [134]. Chromatin domains marked H3K4me3 are involved in attenuation of intact long interspersed nuclear
The composition and chromatin recognition of Polycomb (PcG) and Trithorax (TrxG) complexes in ESCs.

**A.** PcG is composed of Polycomb Repressive Complexes PRC1 and PRC2, which are responsible for methylation on H3K27 and chromatin compaction. PRC2 contains four functional subunits EZH1/2, SUZ12, EED, and RbBP4. PRC1 has six distinct PCGF subunits, H2A monoubiquitin ligases RING1A/B and a unique set of PRC1-associated proteins. The mammalian TrxG is composed of SET1/COMPASS and MLL/COMPASS-like complexes containing four members of MLL family, ASH2L, RbBP5, WDR5, and DPY30. RNF20 is a component of an E3 ubiquitin-protein ligase complex that mediates monoubiquitination of lysine 120 of histone H2B (H2BK120ub1). The SET1/COMPASS complex initiates a relaxed chromatin configuration by binding to H2BK120ub1. B. EZH2, an enzymatic subunit of PRC2, initiates trimethylation on H3K27 over the PRC2-bound regions, whereas PRC1 selectively binds to H2AK119ub1. PRC1-linked H2A monoubiquitylation is sufficient to recruit PRC2 to chromatin in vivo, suggesting a mechanism through which recognition of unmethylated CpG islands determines the localization of both PRC1 and PRC2 at the target sites. In the presence of PRC2, PRC1 retains the capacity to occupy the H3K27me3-enriched nucleosomes associated with poised RNA Polymerase II (Pol II), which is phosphorylated at serine 2. The TrxG-specific complex SET1/COMPASS exhibits a more robust H3K4 trimethylation activity than MLL/COMPASS-like complex. In pluripotent stem cells, CFP1 and MLL1/2 are implicated in targeting SET1/COMPASS to CpG islands, thus playing a critical role in H3K4me3 accumulation. ASH2L recognizes H2BK120ub1 to initiate the recruitment of SET1/COMPASS, which then binds activated Pol II, which is phosphorylated at serine 5, augmenting H3K4me3 domain co-transcriptionally.
elements in pluripotent stem cells [135]. G9a and GLP belong to another group of H3K9 methyltransferases that interact with the MAX repressor complex [136]. In ESCs, MAX knockdown decreases H3K9me2 over the cis-regulatory elements of germ cell-specific genes. G9a and GLP recruit DNA methyltransferases and LSH, a member of the SNF2 family of chromatin remodeling ATPases, to promote efficient and stable gene repression [137]. Loss of LSH significantly reduces DNA methylation across multiple genomic clusters, thereby altering transcriptional output of targeted loci [138]. The G9a-mediated deposition of H3K9me2 at active enhancers causes transcriptional silencing of developmental genes [139]. In addition to H3K9 methylation, G9a has been shown to control gene expression through the H3K27me3-dependent repressive mechanism [140]. The G9a/GLP complex protects imprinted DNA methylation by recruiting de novo DNA methyltransferases, which antagonize TET dioxygenase-dependent erosion of DNA methylation of imprinting control regions [141].

SETD2, the H3K36-specific HMT, guards differentiation of murine ESCs toward the primitive endodermal lineage via the Fgf3-Erk signaling pathway [142]. The tethering of SETD2 by the antisense RNA Tsix is responsible for the H3K36me3-mediated repression of Xist on the X chromosome [143]. The removal of Tsix and PRC2 activities causes a rapid and profound upregulation of Xist and X chromosome repression in male ESCs. SETD7 is implicated in silencing delays of pluripotency genes and induction of developmental genes [144]. Methylation of the linker histone H1 by SETD7 can perturb chromatin structure via reduced deposition of H1 at the OCT4 and NANOG genomic loci during human ESC differentiation.

The extended stretches of H3K4me3 are known as buffer domains, which define promoters of cell type-specific genes [145,146]. The buffer domains support transcriptional consistency, thereby ensuring transcriptional precision at key cell-identity genes. The SET1/COMPASS and COMPASS-like complexes are implicated in the establishment of the H3K4me3-riched domains to counteract the PcG-mediated gene silencing [70,124,147]. The mammalian SET1/COMPASS and MLL/COMPASS-like complexes contain four members of MLL family, namely ASH2L, RbBP5, wDR5, and DPY30 (Figure 4A). The SET1/COMPASS complex binds chromatin through H2BK120u1, a specific ubiquitinilation mark on histone H2B. Due to this epigenetic modification, SET1/COMPASS exhibits a more robust H3K4 trimethylation activity than MLL/COMPASS-like complex, an essential modulator of the long-term genomic memory implicated in cell cycle, senescence, DNA damage, and stem cell biology [148,149]. In the presence of DPY30, TrxG retains the monomethyltransferase activity but exhibits differential di- and trimethylation activities [150]. MLL2 is one of the key factors for establishing and maintaining the methylation-free state at CpG island promoters of active genes [151]. MLL3 contributes to the maintenance of transcriptionally active genome enriched in H3K4me1/K27ac. The cooperation between MLL3 and H3K27 demethylase UTX was proposed to be important in the transition from poised to active enhancers [152]. It was demonstrated that MLL3/MLL4 are major regulators of H3K4me1 at the enhancers in human HCT116 cells and mouse ESCs [153]. Remarkably, in myoblasts, the promoter regions of muscle and inflammatory response genes, where MLL3 and MLL4 initiate H3K4me1 extension, undergo the conditional repression [154]. Interestingly, in embryonic fibroblasts, ESCs and macrophages, the genomic clusters enriched in H3K27me3 and H4K20me1 are also marked with H3K4me1. It has been proposed that epigenetic alterations of this kind are sufficient for establishing condensed chromatin and transcriptional attenuation [154].

The conserved subunit CFP1 is implicated in targeting SET1/COMPASS to CpG islands (Figure 4B). In mouse ESCs, CFP1 is instrumental for H3K4me3 accumulation, which in turn contributes to genome-wide H3K9 acetylation [155]. Therefore, deposition of H3K4me3 at gene promoters is dependent on SET1/CFP1 complex augmenting histone acetyltransferase recruitment and overall H3K9 acetylation dynamics.

Although H3K4me3/K27me3 bivalency is required for keeping developmental genes poised for transcriptional activation [122,124], a different type of bivalent signature has been described by Matsumura et al. [156]. In developing preadipocytes and upon lineage commitment of mesenchymal stem cells, H3K4me3/K9me3 bivalent structure (Figure 5A) has been shown to play an important role maintaining adipogenic regulatory genes in a poised state, prior to receiving the proper activation signal [156].

**Histone Demethylation**

Histone demethylase LSD1 (also known as KDM1A), a part of the CoREST repressive complex, facilitates the removal of mono- and di-methyl groups from H3K4me2 [103]. LSD1 interacts with bivalent chromatin (Figure 5B) to sustain the balance between H3K4me2 and H3K27me3 at key developmental genes [157]. Knockout of Lsd1 in mouse ESCs leads to increased acetylation at H3K56, and deregulation of essential regulatory genes indicating that demethylation of H3K4me2 and acetylation of H3K56 are tightly regulated processes during mouse development [82,158]. LSD1 interacts with HDAC1 to facilitate deacetylation of H4K16ac, an important epigenetic mechanism to ensure the pluripotency of ESCs [159]. While LSD1 is enriched at poised promoters, LSD2/KDM1B, a closely related paralog, tends to associate with the genome undergoing active transcription [160]. LSD2 localizes specifically to euchromatin forming an active complex with G9a and NSD3 lysine methyltransferases.

KDM2B, the H3K36-specific histone demethylase, preferentially binds to CpG islands (Figure 4B). KDM2B
facilitates the establishment of H2AK119ub1-positive chromatin in eSCs by recruiting RING1B/PRC1 [130-132,161]. Depletion of KDM2B causes de-repression of lineage-specific genes and induction of the early stage differentiation followed by loss of H2AK119ub1. KDM2B protects the PcG-occupied promoters against ectopic de novo methylation; its loss leads to a progressive gain of DNA methylation [162]. Pedersen et al. [163] reported that erase of the promoter-enriched H3K9me3 by KDM4 demethylase represents a novel mechanism ensuring transcriptional competence and stability of the pluripotent cell identity. The conditional inactivation of Kdm4a, Kdm4b

**Figure 5.** Bivalent chromatin and the role of non-coding RNAs in the regulation of gene expression. **A.** Two different forms of bivalent domain exist in ESCs and mesenchymal stem cells (MSCs). In pluripotent stem cells, H3K4/H3K27me3 bivalency pauses Pol II at promoters of developmental genes. In MSCs and preadipocytes, deposition of a repressive mark H3K9me3 by SETDB1 pauses Pol II at promoters. DNA methylation contributes to the formation of the H3K4/H3K9me3 bivalent domain by facilitating the recruitment of the repressive complex MBD1/MCAF1/SETDB1 over the gene bodies, thus restricting the differentiation potential of preadipocytes. Loss of H3K9me3 in adipocytes enables Pol II elongation at the developmental genes such as Cebpa and Pparg. **B.** Physical contact between IncRNAs and TrxG complex can promote transcriptional activation. WDR5, a subunit of the SET1/COMPASS complex, binds to the IncRNA HOTTIP transcribed from the HOXA locus. The interaction between WDR5 and HOTTIP elicits gene activation via MLL-mediated H3K4 trimethylation. As an epigenetic repressor, IncRNA HOTAIR ensures the recruitment of PRC2 and LSD1 at HOXD cluster to initiate accumulation of H3K27me3 and demethylation at H3K4me3, thereby enforcing a silent chromatin state.
and Kdm4c alleles in mouse ESCs revealed that while individual family members are dispensable for stem cell maintenance and embryogenesis, combined deficiency of Kdm4a and Kdm4c causes early embryonic lethality and impaired ESC self-renewal. KDM4B and KDM4C localize to the H3K4me3-positive promoters, where they have widespread and redundant roles in preventing accumulation of H3K9me3 and H3K36me3 [163]. The functional interaction between KDM5A and E2F4 underlies the spreading of a repressive chromatin structure in the cell cycle genes [164]. KDM5B can bind to PRC2 and LSD1 to coordinate reduction of H3K4me3 at active promoters, while its co-occupancy with MRG15 at the H3K36me3-rich clusters is implicated in staging the repressive state [165,166]. Depletion of KDM5B results in an increase in H3K4me3 and cryptic transcription, although transcriptional elongation of genes bound by KDM5B is prevented [166]. There has been evidence that KDM5C augments discrimination between enhancers and core promoters [167]. During neural differentiation, occupancy of KDM5C at gene promoters attenuates gene expression, while it’s binding to active enhancers stimulates transcription. The reduced trimethylation of H3K27 at the promoter and initiation of the wnt-dependent Brachyury promoter and initiation of the Wnt-dependent mesoderm differentiation through the recruitment of β-catenin to specific regulatory elements is mediated by KDM6A and KDM6B [168-170].

Efficient differentiation of mesodermal lineage cells from mouse ESCs is dependent upon histone demethylase PHF8 [171]. Functionally, PHF8 facilitates transcription of genes critical in programmed cell death. For instance, a repressive histone mark H3K9me2, once erased with PHF8, can trigger transcriptional activation of a pro-apoptotic gene Pmaip1.

Histone Ubiquitylation

RNA polymerase II phosphorylated at serine 2 residue associates with genes undergoing active transcription, whereas serine 5-phosphorylated Pol II is a hallmark of poised RNA polymerase II, which binds to the silenced genes [172,173]. RING1B, the catalytic subunit of PRC1, enforces the poised Pol II configuration by histone H2A ubiquitylation [174]. H2A monoubiquitylation spreads at unmethylated CpG-rich regions (Figure 4B) and is sufficient for PRC2 recruitment in vivo [128]. KDM2B, functioning as a subunit of the noncanonical PRC1, is required for accumulating H2AK119ub1-positive domain in mouse ESCs [131,132]. It has been shown that KDM2B preferentially binds to CpG islands, thereby playing a key role in PRC1 recruitment to its target genes (Figure 4B). Another report suggested that the expression of lineage-specific genes is blocked by MDM2 oncprotein, which binds to EZH2 increasing abundance of H3K27me3 and H2AK119ub1 at the Pcg-bound regions [175].

Dynamic changes in histone ubiquitylation are initiated and maintained in a timely and well-coordinated manner during ESC differentiation [176]. For instance, monoubiquitylation of histone H2B on lysine 120 (H2B K120ub1), which is catalyzed by the E3 ligase RNF20, was reported to be necessary for transcriptional induction of relatively large genes [177]. H2B K120ub1 marks genes required for lineage commitment of ESCs (Figure 4B) and therefore, the reduction of H2B ubiquitylation triggers further alterations along lineage-specific pathways. There is evidence that H2B K120ub1 is critical for maintaining multipotency of human mesenchymal stem cells [178].

Upon ESC differentiation, the deubiquitinase USP44, a negative regulator of H2B ubiquitylation, is downregulated, thereby contributing to an increase in H2B K120ub1 [176]. The level of ubiquitination on H2A is controlled by USP16, another histone deubiquitinase [179].

Epigenetic Readers: Bromodomains and Chromodomains

While histone acetyltransferases are considered to be epigenetic writers, bromodomains (BRDs) serve as epigenetic readers participating in fine-tuning gene expression (Figure 2). The BET (bromodomain and extra-terminal) family of BRDs binds to an acetyl-lysine residue on histone tails [180]. Hence, BRDs occupy super-enhancers, large genomic clusters enriched in H3K27ac, p300, and Mediator, an essential epigenetic signature for dynamic and coordinated regulation of key cell identity genes [181]. The core transcription factors OCT4, SOX2, and NANOG are enriched in super-enhancers, whereas in pluripotent cells undergoing differentiation, BRD-bound super-enhancers serve as a scaffold for lineage-specific master regulators [182,183]. Recent work by Devaiah et al. have shown that BRD4 functions as histone acetyltransferase by acetylating H3K12, an intermediate step augmenting nucleosome depletion, chromatin relaxation and transcriptional activation of cognate genes [184]. H3K122 is known as a molecular target of p300/CBP and its acetylation is associated with an array of actively transcribed genes [79].

The chromodomain protein CDYL acts as an epigenetic reader by recognizing chromatin enriched in H3K9me2 and H3K27me3 as part of a repressive complex assembled at the inactive X chromosome in mouse ESCs [185]. This event, in turn, facilitates anchoring of G9a and H3K9me2 deposition at target loci. CHD1, one of nine members of the chromodomain helicase family, is also necessary for mouse ESC pluripotency [186]. CHD1 binds to active genes enriched in H3K4me3 as part of the preinitiation complex. Mediator recruits CHD1 to control the preinitiation complex assembly [186]. CHD7 associates with active enhancers to modulate ESC-specific gene expression [187]. Most of the CHD7-bound sites co-localize with p300 and H3K4me1. MRG15, another protein with chromodomain, was shown to assist KDM5B in
binding to the H3K36me3-positive nucleosomes in mouse eSCs [166].

**DNA Methylation**

DNA methylation, a transfer of the methyl group from S-adenosylmethionine to a cytosine, has a profound impact on chromatin structure, gene expression and maintenance of cell-type identity [188]. DNA methylation predominantly occurs at CpG islands (CGIs), one of the most prominent features of the mammalian genome [69]. CGIs retain the GC-rich base composition and high density of CpG dinucleotides. It was estimated that a great majority of human genes initiate transcription from CGIs that mark gene promoters [189,190]. The bivalent and active promoter regions are enriched in CpG, while H3K27me3-only promoters lack CpG islands [191]. Methylation of DNA at the fifth position of cytosine (5mC) in CpG dinucleotides is commonly associated with gene repression. Moreover, 5mC is enriched in exons and at intron-exon junctions, suggesting a specific role for DNA methylation in the crosstalk between elongation and RNA splicing [192].

DNMT1, DNMT3A, and DNMT3B are three conserved DNA methyltransferases that are responsible for cytosine methylation [69]. The initial stages of de novo methylation require the cooperative interaction between DNMT3A and DNMT3B, while completion of DNA methylation requires DNMT1 and G9a [193]. The repressed state of the non-transcribed CpG-rich genes that control cell identity is maintained by PRC2 [194]. Remarkably, the acquisition of H3K27me3 is accompanied by loss of DNA methylation [195]. Conversely, DNA hypomethylation triggers the eviction of PRC2 and loss of H3K27me3 causing ectopic expression of cognate genes. Surprisingly, in innate myeloid cells, TET2 is able to recruit HDAC2 with the assistance of IκBτfactor to initiate the gene-specific transcriptional repression [206]. The TET-mediated DNA hydroxymethylation is critical for de novo establishment and maintenance of H3K4me3-rich active domains marking CGIs, while TET binding to PRC2 contributes to the establishment of H3K27me3-positive repressive chromatin. Methyl CpG-binding proteins (MBDs) control expression of pluripotency genes in ESCs by associating with the HDAC/NuRD repressive complex.

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**Figure 6**. The close ties between DNA methylation and histone modification. Methylation of cytosine by DNA methyltransferases (DNMTs) establishes repressive chromatin, which is stabilized by PRC2 and NuRD complexes. However, in some cancer cells, DNA methylation ensures structural integrity of the H3K27ac-rich enhancers [199]. The TET-mediated DNA hypomethylation triggers the eviction of PRC2 and loss of H3K27me3 causing ectopic expression of cognate genes. Surprisingly, in innate myeloid cells, TET2 is able to recruit HDAC2 with the assistance of IκBτfactor to initiate the gene-specific transcriptional repression [206]. The TET-mediated DNA hydroxymethylation is critical for de novo establishment and maintenance of H3K4me3-rich active domains marking CGIs, while TET binding to PRC2 contributes to the establishment of H3K27me3-positive repressive chromatin. Methyl CpG-binding proteins (MBDs) control expression of pluripotency genes in ESCs by associating with the HDAC/NuRD repressive complex.
eight-cell stage embryos exhibiting a characteristic phenotype of holoprosencephaly [204]. Expression of genes of cholesterol biosynthesis is reduced in Tet1/3 knockout blastocysts. Inactivation of all three Tet paralogs leads to a complete loss of 5hmC and impaired ESC differentiation suggesting that the TET-mediated DNA demethylation is an essential epigenetic switch to ensure proper cell function [205]. Surprisingly, TET2 is able to recruit HDAC2 to initiate the gene-specific transcriptional repression (Figure 6) using the histone deacetylation pathway [206]. The transcriptional repressor REST supports TET3 binding to chromatin, thereby influencing 5hmC generation, while the ability of TET3 to interact with NSD3 induces H3K36me3 marks in mouse retina [207]. Recently, Zeng et al. discovered that RNA-binding protein Lin28 recruits TET enzymes to genomic loci to initiate 5mC to 5hmC conversion [208]. In the vertebrate genome, a widespread DNA demethylation of enhancers occurs during the phylotypic period [209]. The recruitment of TET enzymes to 5mC and enrichment of 5hmC at enhancers implicates DNA demethylation in the activation of lineage-specific genes. Loss of TET paralogs in Danio leads to the reduced chromatin accessibility and an increase in DNA methylation at tissue-specific enhancers [209]. In addition, TET enzymes modulate sub-telomeric methylation levels, thereby playing a critical role in chromosomal stability [210]. Depletion of all three Tet paralogs in mouse ESCs leads to increased frequency of telomere loss and chromosomal fusion. Mechanistically, depletion or deficiency of Tet genes increases DNMT3B and decreases 5hmC levels, resulting in elevated levels of DNA methylation at sub-telomeres [210]. The critical role of TETs in regulating the crosstalk between DNA methylation and histone methylation at CpG-rich bivalent promoters was recently revealed by Kong et al. [211]. Their study showed that TET2-mediated DNA demethylation is required for de novo establishment and maintenance of H3K4me3/K27me3 bivalency at CGIs. In human epidermal stem cells, the TET2-dependent association of DNMT3A with p63 is required for high-level maintenance of 5mC at the center of enhancers, whereas DNMT3B sustains DNA methylation along the entire enhancer length [212]. Given the fact that active enhancers are co-occupied by DNMT3A and DNMT3B and this type of interaction is required for enhancer RNA production, this is a surprising discovery.

Methyl CpG-binding protein family (MBD) represents a set of epigenetic readers that share a conserved methyl-CpG domain [213]. Almost all MBD proteins, with the exception of MBD3, are able to bind specifically to the high-density CpG promoters [214]. MBD3, as part of the NuRD repressive complex, binds to the regulatory sequences occupied by HDAC1/NuRD (Figure 4B) controlling expression of Oct4, Nanog, and Klf4 in ESCs [107].

Non-coding RNAs

The discovery of a large number of non-coding RNAs (ncRNAs) forming a powerful RNA surveillance system has redefined fundamental principles of gene regulation in stem cells, including ESCs, induced pluripotent stem cells, mesenchymal stem cells, and adult stem cells [215]. Knowledge of non-coding RNAs and their function in pluripotency, self-renewal and differentiation has the potential to reveal novel molecular cues involved in induction and maintenance of the pluripotent state, as well as clinically relevant cell types and tissues [216].

Long ncRNAs (lncRNAs) refer to a heterogenic class of RNAs that includes intergenic lncRNAs, enhancer RNAs (eRNAs) and antisense transcripts [217,218]. Small ncRNAs navigate the Argonaute complex to nascent RNA templates to facilitate the recruitment of histone methyltransferases and DNA methyltransferases, while lncRNAs participate in transcriptional silencing via independent mechanisms [219]. The expression profile of lncRNAs is often tissue- or cell type-specific, including lncRNAs that have been linked to embryonic and adult stem cells [220]. Bogu et al. discovered that lncRNAs typically associate either with the promoters or enhancers in various mouse tissues [221].

The non-coding RNA Gm15055 represses Hoxa genes in mouse ESCs [222]. Gm15055 maintains H3K27me3 enriched chromatin over the Hoxa cluster by recruiting PRC2 in mouse ESCs. On the contrary, Hottip, a lincRNA transcribed from the 5′ tip of the human HOXA locus, coordinates the activation of several 5′ HOXA genes [223]. Hottip RNA anchors the COMPASS complex across the HOXA locus via binding to WDR5 (Figure 5B), an essential intermediate step for initiating H3K4me3 accumulation and transcriptional activation. In mammals, inactivation of X-chromosome is mediated by lncRNA transcript known as Xist, which binds along the inactive X chromosome [224]. Xist induces posttranslational histone modifications and DNA methylation to achieve a stable repression of all X-linked genes throughout development and adult life. In different study, ablation of mouse Hotair lncRNA was found to be critical in homeotic transformation of skeletal elements [225]. It has been shown that Hotair binding to Hoxd genes ensures the recruitment of PRC2 and LSD1 (Figure 5B), initiates trimethylation at H3K27 and demethylation at H3K4me3, thereby enforcing chromatin compaction. During differentiation of hematopoietic lineages, long ncRNA HoxBlinC binds to the mouse Hoxb loci to facilitate the recruitment of SETD1A/MLL1 complexes [226]. This event triggers transcriptional activation of genes in the Hoxb cluster through long-range chromatin interactions.

There is growing evidence that lncRNAs participate in the recruitment of PRC2 in the gene dense regions enriched in H3K27me3, H2AK119ub or H3K36me3 [227]. LncRNAs, which are derived from pseudogenes, were shown to be an integral part of the epigenetic surveillance
system [228]. It was demonstrated that the mouse Oct4 pseudogene Oct4P4 is critical for both ESC self-renewal and differentiation. A nuclear-restricted IncRNA, which is produced from the sense Oct4P4 transcription, forms a complex with SUV39H1 to initiate the imposition of H3K9me3 and HP1α to the Oct4 promoter. This regulatory cascade leads to Oct4 silencing and reduced ESC self-renewal [228].

Divergent IncRNAs transcribe in the opposite direction to the nearby protein-coding genes and comprise almost 20 percent of total IncRNAs in the mammalian genome [229]. In pluripotent stem cells, divergent IncRNAs were shown to regulate expression of the neighboring genes. For example, binding of the divergent IncRNA Evx1as to the regulatory region of the neighbor gene EVX1 initiates its transcription during mesendodermal differentiation [229].

MicroRNAs (miRs), a class of small non-coding RNAs 19-24 nucleotides in length, mediate post-transcriptional control of ESC pluripotency and cellular re-programming [230,231]. MicroRNAs play a significant role in vertebrate neural crest development and facial morphogenesis [37,232-234]. A subset of miRs regulates its cognate genes during neurogenesis, chondrogenesis and hematopoiesis [235-237]. During differentiation of smooth muscle cells, miR-22 assists to anchor MeCP2 to chromatin followed by enrichment of H3K9me3 and subsequent inactivation of key developmental genes [238]. miR-24-1 has been linked to transcriptional activation of eRNAs, a regulatory event associated with the recruitment of p300 and Pol II to enhancers [239]. During osteoblast differentiation and bone formation, miR-145 plays an important role in the CBFB-dependent regulation, a molecular process dependent upon the interaction between Runx2 and the core binding factor beta CBFB [240]. Surprisingly, the recruitment of EZH2 and SUZ12 to many bivalent genes requires the miR biogenesis enzyme Dicer [241]. In ESCs depleted of Dicer, miRs are linked with subsequent inactivation of key developmental genes [238]. micR-24-1 has been linked to transcriptional activation of eRNAs, a regulatory event associated with the recruitment of p300 and Pol II to enhancers [239]. During osteoblast differentiation and bone formation, miR-145 plays an important role in the CBFB-dependent regulation, a molecular process dependent upon the interaction between Runx2 and the core binding factor beta CBFB [240]. Surprisingly, the recruitment of EZH2 and SUZ12 to many bivalent genes requires the miR biogenesis enzyme Dicer [241]. In ESCs depleted of Dicer, miRs are linked with subsequent inactivation of key developmental genes [238].

Furthermore, there is evidence for a coordinated crosstalk between IncRNAs and miRs [242,243]. A complex network of transcription factors, chromatin remodeling complexes, miRs and IncRNAs has been implicated in maintaining the balance between self-renewal and multilineage differentiation capacity of pluripotent stem cells [233]. The cytoplasmic IncRNA-RoR is able to trap miR-145, an inhibitor of the core pluripotency master regulators, thereby derepressing the translation of OCT4, SOX2 and NANOG in human ESCs [244]. The primate-specific IncRNAs, such as developmental pluripotency-associated transcripts 2, 3 and 5 (HPAT2, HPAT3 and HPAT5), are frequently clustered at transposable elements [245]. These IncRNAs modulate the function of pluripotent stem cells and the formation of the inner cell mass lineage in the preimplantation embryo. For instance, HPAT5 acts as a key component of the pluripotency network by interacting with the let-7 microRNA [245].

**EPIGENETIC MECHANISMS IN DENTAL PULP STEM CELLS**

Epigenetic alterations have been documented in association with periodontal disease, hypodontia, during enamel development and odontogenic differentiation [5-12,246,247]. The paper by Duncan et al. summarizes research on functional significance of histone deacetylases and DNA methyltransferase inhibition in dental pulp as a promising potential for regenerative endodontic regimens [5]. In DPSCs committed to become mature odontoblasts, dynamic changes in the epigenetic landscape have been linked to permissive chromatin associated with transcriptional upregulation [62]. Despite some progress, relatively little is known about the epigenetic code and consequent pleiotropic effects that drive DPSCs into specialized cell lineages.

**Histone Acetylation and HDAC Inhibition**

Attenuation of HAT activity by garcinol, an inhibitor of histone acetyltransferase p300, can reverse histone acetylation during osteogenesis [248]. The DSPP gene, which is mainly expressed in odontoblasts and pre-ameloblasts, is down-regulated in the garcinol-treated DP cells. There is evidence that at the DSPP loci histone acetylation contributes to odontoblast differentiation and maturation of pulp stem cells [248]. p300 is one of the main HATs in the regulation of core pluripotency network in DPSCs [249]. The augmentation of p300 expression does not affect the ability of stem cells to proliferate, but elevates the expression of NANOG and SOX2. Although p300 exerts very little effect on the odontogenic fate, the expression levels of DMP1, DSPP, DSP, OPN and OCN are profoundly reduced in DP cells [249]. It has been found that p300-mediated histone acetylation can increase the extension of H3K9ac deposition on the promoters of OCN and DSPP [250]. Ablation of p300 initiates the formation of mineralized nodules and ALP activity, while the expression of DMP1, DSPP, and DSP (Table 2) is dramatically reduced in DPSCs undergoing odontogenic differentiation.

Histone deacetylase inhibitors (HDACi) alter the homeostatic balance between histone acetylation and deacetylation, increase the transcriptional rate and influence cell physiology suggesting that inhibition of HDACs has great potential in restorative dentistry [251]. Trichostatin A (TSA) is an organic compound that selectively inhibits the class I and class II HDACs [252]. TSA promotes proliferation and odontoblastogenesis in human DPSCs, accelerates mineral nodule formation and enhances dentin formation and odontoblast differentiation.
during tooth development [253]. TSA increases the expression of PCNA, CCND1, DSP, DMP1, BSP, and OCN and activates the JNK/c-Jun-dependent pathway. The expression of SMAD family and NFIC is also elevated by TSA. The following changes in the postnatal molars such as an increased thickness of dentin and accelerated odonoblast proliferation were observed in mouse embryos treated with TSA [253]. Valproic acid (vPA), a drug primarily used to treat epilepsy and bipolar disorder, in combination with TSA accelerates the reparative processes in dental pulp [254]. HDAC inhibition triggers induction of mineralization and increases expression of DMP1, BMP2/4, Nestin, p53, and DSPP [255]. The suppression of histone deacetylase activity promotes expression of OPN and BMP2, thereby affecting cellular differentiation. It has been shown that VPA can reduce transcription of OCN in DPSCs and osteoblasts, while the expression of OPN and BSP remains elevated [256].

Histone Methylation

The potential application of EZH2 in tissue regeneration including nervous system, muscle, pancreas, and dental pulp has been recently discussed [257]. It was suggested that PRC2 has a major role during DP inflammation, proliferation, and regeneration [258]. Upon inflammatory stimuli, inhibition of the enzymatic activity of EZH2 down-regulates expression of IL-1b, IL-6, and IL-8 in the infected pulp tissue (Table 2) and impedes DP cell proliferation by decreasing cell number, arresting the cell cycle, and increasing apoptosis. The adipogenic induction leads to the suppression of EZH2, which in turn diminishes transcriptional activity of PPAR-r and CEBP/a, whereas alkaline phosphatase activity and expression of Osx and BSP stays elevated [258].

The research conducted by Gopinathan et al. revealed that epigenetic changes play an important role in the terminal differentiation of odontogenic lineages in dental pulp and dental follicle [62]. Compared to DF cells, the expression of pluripotency genes OCT4, NANOG, and SOX2 is higher in DP cells. The authors also observed a substantial increase in H3K27me3 followed by repression of DSSP and DMP1 in the DF, but not in the DP. The histone mark H3K4me3, associated with active genes, is enriched at the promoters of early mineralization genes RUNX2, MSX2, and DLX5 in DF and DP progenitors, while H3K9me3 or H3K27me3, marks of repressive chromatin, are clustered in the vicinity of OSX, IBSP, and BGLAP [62].

Interestingly, the SUMO-specific isopeptidase SENP3 controls the level of H3K4me3 by regulating histone methyltransferase SET1/MLL in human DF stem cells [259]. Removal of SUMO from SET1/MLL by SENP3 is required for activation of key transcription factors such as DLX3 during osteogenic differentiation of DF progenitors. This observation suggests that a similar regulatory framework could also exist in DPSCs.

In another recent study, inhibition of histone demethylase KDM6B by alcohol has been linked to the dysregulation of odontogenic/osteogenic differentiation in DPSCs [260]. The inactivation of KDM6B associates with reduced mineralization of DPSCs, while in the ethanol-treated DPSCs, KDM6B facilitates the restoration of

| Table 2. Epigenetic changes in human and mouse dental pulp stem cells |
|---------------------------------------------------------------|
| **Family**          | **Genomic targets**                      | **References** |
|---------------------|----------------------------------------|----------------|
| **Epigenetic enzymes**                                      |                                         |                |
| p300                | DMP1, DSP, DSP, OPN, OCN               | [249,250]      |
| EZH2                | PPAR-r, CEBP/a, Osx, BSP, IL-1b, IL-6, IL-8 | [258]          |
| KDM6B               | genes implicate in differentiation     | [260]          |
| DNM Ts              | DSSP, DMP1, OSX, RUNX2, DLX5           | [262-266]      |
| TET1                | DSSP, DMP1                            | [268]          |
| **MicroRNA**        |                                         |                |
| hsa-miR-218         | RUNX2                                  | [276]          |
| miR-32              | DSPP                                   | [277,278]      |
| miR-885-5p          | DSPP                                   | [277,278]      |
| miR-586             | DSPP                                   | [277,278]      |
| let-7               | DMP                                    | [277,278]      |
| miR-143             | Dssp, Dmp1                             | [279]          |
| miR-145             | Dssp, Dmp1                             | [279]          |
| miR-720             | DNM Ts, NANOOG                         | [281]          |
| miR-433             | GRB                                    | [282]          |
| has-miR-516a-3p     | WNT5A                                  | [283]          |
| has-miR-7-5p        | EGFR                                   | [283]          |
| miR-152             | SIRT7                                  | [285]          |
| miR-146a            | IRAK1, TRAF6                           | [246]          |
genes involved in odontoblast differentiation. KDM6B have already been implicated in promoting osteogenesis by erasing H3K27me3 from the promoters of odontogenic markers SP7, OCN, and OPN [247].

DNA Methylation

The odontogenic-specific DNA hypomethylation was suggested to be a typical feature of human dental pulp [261]. The toll-like receptors TLR-2 and TLR-4 mediate the activation of immune cells in response to bacterial infection in DP cells. The hypomethylated regions in TLR-2 and in the CD14 gene, which encodes TLR4 co-receptor, have been detected in healthy and inflamed human DP tissues [261].

The DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-aza-CdR) reactivates genes repressed by DNA methylation [262-264]. The 5-Aza-CdR-triggered DNA demethylation significantly inhibits the proliferation of DP cells and up-regulates the expression of DSPP, DMP1, OSX, RUNX2, and DLX5 (Table 2). DNA demethylation elevates the capability of odontogenic differentiation in DP cells suggesting that DNA methylation may play a fundamental role in reparative dentinogenesis in human dental pulp [265]. Similarly, the selective suppression of mouse DP cell differentiation into skeletal muscle cells by 5-Aza-CdR suggests that differential induction of DPSCs is associated with DNA demethylation [266].

The research reported by Yoshioka et al. failed to detect obvious changes in the spatial distribution of 5mC and 5hmC in odontoblasts and DP cells [267]. According to them, DNMT1 was vigorously transcribed, while expression changes of DNMT3A, DNMT3B and members of the TET family were relatively modest. The only exception is TET1, which was shown to exhibit elevated expression in immature dental epithelial cells [267]. Interestingly, TET1 knockdown suppresses the proliferative and odontogenic capacity of human DP cells suggesting that TET1 may play an important role in dental pulp repair and regeneration [268]. The study also revealed that alkaline phosphatase activity, the formation of mineralized nodules, and expression of DSPP and DMP1 were significantly reduced in the TET1-depleted DP cells undergoing odontogenesis.

Non-coding RNAs

MicroRNAs and long non-coding RNAs have an essential function in inflammation and immunity, the key processes associated with pulp pathology [10,269-271]. Many miRs are predicted to target genes linked to multiple biological pathways including the mitogen-activated protein kinase (MAPK) and Wnt signaling, proinflammatory cytokines and other key mediators of the immune and inflammatory response [272,273]. miR-181a binds 3'UTR of IL-8 keeping its expression at elevated levels (Table 2) [274]. Kong et al. suggested that Oct4B1, an essential functional isoform of Oct4, by interacting with miRs, contributes to the inflammatory response in DP cells [275]. Upon bacterial lipopolysaccharide (LPS) stimulation, the expression dynamics of Oct4B1 and Oct4B2 are elevated in DP cells, whereas knockdown of Oct4B1 leads to increased apoptosis. MiRs that associate with the MAPK, Wnt and Toll-like signaling pathways are differentially regulated after Oct4B1 knockdown [275].

In undifferentiated DP cells, hsa-miR-218 down-regulates RUNX2 transcription, whereas during DP differentiation, decrease in hsa-miR-218 causes up-regulation of RUNX2 [276]. The post-transcriptional control of DSSP is achieved via miR-32, miR-885-5p and miR-586, while DMP regulation is mediated by the Let-7 microRNA precursor [277,278]. miR-143 and miR-145 control the odontoblast lineage and dentin formation through the Klf4/Osx-dependent regulatory mechanisms. Down-regulation of miR-145 and miR-143 promotes odontoblast differentiation and increased expression of Dssp and Dmp1 in mouse primary DP cells [279]. First, the recruitment of Klf4 and Osx to the Dssp and Dmp1 promoters leads to transcriptional activation of both genes and odontoblast differentiation; second, miR-145 binds to the 3'UTRs of Klf4 and Osx to inhibit their expression; third, Klf4 recruitment to the miR-143 regulatory region suppresses miR-143 transcription; fourth, miR-143 mediates odontoblast differentiation, at least in part, through the miR-145-controlled mechanism [279]. Additionally, miR-143 together with miR-135, induces myogenic differentiation of DPSCs [280]. The expression pattern of NANOG and DNA methyltransferase genes is controlled by miR-720 [281]. Conversely, GRB2 and the RAS-MAPK signaling pathways are regulated by miR-433 [282]. It was documented that the reduction of proliferative and mineralization abilities as well as increased cell death of DP cells is mediated miR-433. Vasanthan et al. reported that hsa-miR-516a-3p and hsa-miR-7-5p, two highly expressed micRNAs in dental pulp, impose differential control on target genes [283]. Inactivation of hsa-miR-516a-3p induces WNT5A expression, while knockdown of hsa-miR-7-5p increases EGFR expression. Another microRNA, miR-424, plays a negative role in the regulation of the human pulp endothelial differentiation; the attenuation of miR-424 was proposed to contribute to dental pulp repair and regeneration [284]. During DPSC senescence, upregulation of miR-152 decreases the level of SIRT7 expression, thus influencing the histone acetylation status in DPSCs [285]. Among developmental regulators, LPS is essential for pulp pathogenesis; it can increase DP cell migration using the regulatory pathway controlled by miR-146a-TRAF6/IRAK1 [246]. Upon LPS induction, activated miR-146a facilitates the migration of pulp cells, whereas the expression of IRAK1 and TRAF6 is significantly exacerbated.

Chen et al. have documented that Wnt/β-catenin signaling can be controlled by a member of lncRNAs during DP differentiation [286]. Upregulation of lncRNA
DANCR leads to a significant decrease in the expression levels of p-GSK-3β and β-catenin indicating that lncRNA DANCR imposes inhibitory dynamics on the activation of the Wnt/β-catenin signaling pathway during odonto-blast differentiation [286].

**CHROMATIN TOPOLOGY**

Transcription factors and chromatin remodeling complexes establish and maintain cell identity via global genomic changes. In the 3D nuclear space, the tissue-specific distal enhancers are typically located in close physical proximity to promoter regions through the formation of chromosome loops that can range from large-scale folding of whole chromosomes to smaller genomic segments [51-56]. Discrete looping interactions determine the differentiation potential of a cognate stem cell into more specialized cells such as odontoblasts. Therefore, the functional specificity of chromatin topology governing cell-state transitions should provide valuable insight into the regulatory framework underlying different phases of tooth organogenesis. It is also critical to understand how craniofacial osteogenesis generates the phenotypic variation within a population, thereby rendering the raw material for evolution. It has been shown that variations in the highly conserved regulatory pathway controlling brain development underlie a morphological difference between the upper and lower jaws in avian species [287]. In this respect, enhancer-promoter interaction networks involved in early neurogenesis are highly sensitive to chromatin structural changes [50]. Recently, the study by Emera et al. mapped enhancers required for development of the mammalian neocortex in close proximity to genes associated with cell migration, axon guidance, and cell signaling [288]. The proposed model suggests that relatively simple cis-regulatory elements emerging from the genomic background are able to accumulate H3K27ac, and some elements ultimately evolve into more complex enhancers, whereas others are lost during evolution. Morphogenesis of the face and brain is intrinsically linked by the dynamics of regulatory crosstalks between key developmental genes. Signals from neural crest cells, for instance, regulate expression of Fgf8, which controls growth of the anterior forebrain, while the Shh signaling pathway is
implicated in head morphogenesis including neuroectoderm of the ventral forebrain, facial ectoderm and pharyngeal endoderm [289,290].

In fact, over past few years, the rapid progress in next-generation sequencing and advanced chromatin mapping technologies [291-294] has opened new opportunities for further interrogation of chromatin topology and assessment of proper temporal and spatial differences in the control of gene expression during orofacial morphogenesis. Integrative analysis from transcriptomics, epigenomic and proteomic data can significantly increase our ability to uncover regulatory wirings and intricate 3D communications that underlie odontogenic lineage commitment (Figure 7).

CONCLUSIONS AND OUTLOOK

The self-renewal capacity and potential to differentiate into multiple cells makes ESCs very attractive for regenerative medicine. Using advances in gene editing and 3D tissue engineering technologies, it became possible to reproduce self-organizing qualities of embryonic and adult stem cells [295]. Based on such properties of stem cells, diverse multi-cellular tissues, also known as organoids, could be re-created, although the current technology is facing some inherent limitations such as the control of the cell type, cell organization, and cell-cell/cell-matrix interactions [296]. How epigenetic changes influence chromatin features, gene expression and genome stability, and thus contribute to lineage commitment and stem cell differentiation will remain as one of the main challenges facing modern biology. Therefore, the genome-wide strategies are going to play an increasingly important role in therapies that aim to potentiate tissue regeneration and repair. The technological advances in high-resolution imaging and the development of new methods such as chromatin proteomic profiling (ChIP-MS) and quantitative interaction proteomics have established a platform for identification of protein complexes associated with regulatory sequences carrying specific histone tags [297-299]. As we become more aware of complex context-specific relationships between the epigenome and environmental fluctuations during oral development [300], the necessity to challenge long-standing paradigms is now widely acknowledged. Systems biology has the potential to unravel the essential coding principles that constitute the osteogenic capacity of different progenitor populations. A systematic approach to an in-depth understanding of epigenetic determinants in dental pulp would ultimately create important clinical implications, and can possibly reveal the causes of rare and common oral diseases.

REFERENCES

1. Zoghbi HY, Beaudet AL. Epigenetics and Human Disease. Cold Spring Harb Perspect Biol. 2016;8(2):a019497.
2. Hamidi T, Singh AK, Chen T. Genetic alterations of DNA methylation machinery in human diseases. Epigenomics. 2015;7(2):247-65.
3. Luo Z, Lin C. Enhancer, epigenetics, and human disease. Curr Opin Genet Dev. 2016;36:27-33.
4. Etchebarry JP, Mostoslavsky R. Interplay between Metabolism and Epigenetics: A Nuclear Adaptation to Environmental Changes. Mol Cell. 2016;62(5):695-711.
5. Duncan HF, Smith AJ, Fleming GJ, Cooper PR. Epigenetic modulation of dental pulp stem cells: implications for regenerative endodontics. Int Endod J. 2016;49(5):431-46.
6. Martins MD, Jiao Y, Larsson L, Almeida LO, Garaeoa-Pazmino C, Le JM, et al. Epigenetic Modifications of Histones in Periodontal Disease. J Dent Res. 2016;95(2):215-22.
7. Fan Y, Zhou Y, Zhou X, Xu X, Pi C, Xu R, et al. Epigenetic Control of Gene Function in Enamel Development. Curr Stem Cell Res Ther. 2015;10(5):405-11.
8. Larsson L, Castillo RM, Giannobile WV. Epigenetics and Its Role in Periodontal Diseases: A State-of-the-Art Review. J Periodontol. 2015;86(4):556-68.
9. Seo JY, Park YJ, Yi YA, Hwang JY, Lee IB, Cho BH, et al. Epigenetics: general characteristics and implications for oral health. J Healthc. 2016;40(1):14-22.
10. Hui T, Wang C, Chen D, Zheng L, Huang D, Ye L. Epigenetic regulation in dental pulp inflammation. Oral Dis. 2016; [Epub ahead of print].
11. Wang J, Sun K, Shen Y, Xu Y, Xie J, Huang R, et al. DNA methylation is critical for tooth agenesis: implications for sporadic non-syndromic anodontia and hypodontia. Sci Rep. 2016;6:19162.
12. Schulz S, Immel UD, Just L, Schaller HG, Gläser C, Richter S. Epigenetic characteristics of inflammatory candidate genes in aggressive periodontitis. Hum Immunol. 2016;77(1):71-5.
13. Ledesma-Martínez E, Mendoza-Núñez VM, Santiago-Osorio E. Mesenchymal Stem Cells Derived from Dental Pulp: A Review. Stem Cells Int. 2016;2016:4709572.
14. Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med. 2015;9(11):1205-16.
15. d’Aquino R, De Rosa A, Laino G, Caruso F, Guida L, Rullo R, et al. Human dental pulp stem cells: from biology to clinical applications. J Exp Zool B Mol Dev Evol. 2009;312(5):408-15.
16. Casagrande L, Cordeiro MM, Nör SA, Nör JE. Dental pulp stem cells in regenerative dentistry. Odontology. 2011;99(1):1-7.
17. Groves A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kernte J, et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev. 2008;17(6):1175-84.
18. La Noce M, Mele L, Tirino V, Paino F, De Rosa A, Naddeo P, et al. Neural crest stem cell population in craniomaxillofacial development and tissue repair. Eur Cell Mater. 2014;28:348-57.
19. Aurrekoetxea M, Garcia-Gallastegui P, Irastorza I, Larruquiar I, Uribe-Etxebarria V, Linda F, et al. Dental pulp stem cells as a multifaceted tool for bioengineering and the regeneration of craniomaxillofacial tissues. Front Physiol. 2016;5:289.
20. Aly LA. Stem cells: Sources, and regenerative therapies in dental research and practice. World J Stem Cells. 2015;7(7):1047-53.
21. Maxim MA, Soritau O, Baciu M, Bran S, Baciu G. The role of dental stem cells in regeneration. Clujul Med. 2015;88(4):479-82.
22. Graziano A, d’Aquino R, Laino G, Papaccio G. Dental pulp stem cells: a promising tool for bone regeneration. Stem Cell Rev. 2008;4(1):21-6.
23. Kadar K, Kiraly M, Porcsalmy B, Molnar B, Racz GZ, Blazsek J, et al. Differentiation potential of stem cells from human dental origin - promise for tissue engineering. J Pharmacol Pharmacol. 2009;60 Suppl 7:167-75.
24. Yang J, Yuan G, Chen Z. Pulp Regeneration: Current Approaches and Future Challenges. Front Physiol. 2016;7:58.
25. Potdar PD, Jethmalani YD. Human dental pulp stem cells: Applications in future regenerative medicine. World J Stem Cells. 2015;7(5):839-51.
26. Arthur A, Rychkov G, Shi S, Koblar SA, Grontos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells. 2008;26(7):1787-95.
27. Kawashima N. Characterisation of dental pulp stem cells: a new horizon for tissue regeneration? Arch Oral Biol. 2012;57(11):1439-58.
28. Nosrat Iv, Smith CA, Mullally P, Olson L, Nosrat CA. Dental pulp stem cells respond to cues from the rat retina and differentiate to express the retinal neuronal marker rhodopsin. Neuroscience. 2014;280:142-55.
29. Chang CC, Chang KC, Tsai SJ, Chang HH, Lin CP. Neurogen differentiation of dental pulp stem cells to neuron-like cells is promoted by an extracellular matrix and motor neuronal inductive media. J Formos Med Assoc. 2014;113(12):956-65.
30. Bray AF, Cevallos RR, Gazarian K, Lamas M. Human dental pulp stem cells respond to cues from the rat retina and differentiate to express the retinal neuronal marker rhodopsin. Neuroscience. 2014;280:142-55.
31. Syed-Picard FN, Du Y, Lathrop KL, Mann MM, Funderburgh JL. The epigenetic modifier DNMT3A may contribute to neural crest state: control of stem cell attributes by gene regulation in neural crest development. Dev Biol. 2012;366(1):10-21.
32. Ake S, Hamada K, Miura M, Yamaguchi S. Neural crest stem cell property of apical pulp cells derived from human developing tooth. Cell Biol Int. 2012;36(10):927-36.
33. Janebodin K, Horst Ov, Ieronimakis N, Balasundaram G, Simões-Costa M, Bronner ME. Establishing neural crest progenitor populations. Stem Cells Dev. 2013;22(12):1763-70.
34. Prasad MS, Sauka-Spengler T, LaBonne C. Induction of the neural crest state: control of stem cell attributes by gene regulation during neural crest development. Cell Biol Int. 2012;36(10):927-36.
35. Van der Kooy D, van der Kooy D, van der Kooy D, van der Kooy D, van der Kooy D. Genetic Tailors: CTCF and Cohesin Shape the Genome During Evolution. Trends Genet. 2016;32(4):225-37.
36. Dekker J, Mirny L. The 3D Genome as Moderator of Chromosome Architecture and Function by Polycomb Proteins. Cell. 2016;164(6):1110-21.
37. Groves PM, Brenner M. Dental Pulp Cells Provide Neurotrophic Support for Dopaminergic Neurons and Differentiate into Neurons In Vitro: Implications for Tissue Engineering and Repair in the Nervous System. Eur J Neurosci. 2004;19(9):2385-98.
38. Mohanty V, Shah A, Allender E, Siddiqui MR, Monick S, Roadmap epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015;518(7539):317-30.
39. Perez-Campo FM, Riancho JA. Epigenetic Mechanisms Regulating Mesenchymal Stem Cell Differentiation. Curr Genomics. 2015;16(6):368-83.
40. Fagnocchi L, Mazzoleni S, Zippo A. Integration of Signalling Pathways with the Epigenetic Machinery in the Maintenance of Stem Cells. Stem Cells Int. 2016;2016:8652748.
41. Peng P, Chen QM, Hong C, Wang CY. Histone methyltransferases and demethylases: regulators in balancing osteogenic and adipogenic differentiation of mesenchymal stem cells. Int J Oral Sci. 2015;7(4):197-204.
42. Mohan KN. Stem Cell Models to Investigate the Role of DNA Methyltransferase Machinery in Development of Neuropsychiatric Disorders. Stem Cells Int. 2016;2016:4379425.
43. Vicente R, Noël D, Pers YM, Apparailly F, Jorgensen C. Deregulation and therapeutic potential of microRNAs in arthritic diseases. Nat Rev Rheumatol. 2016;12(4):211-20.
44. Philips-Cremins JE, Sauria ME, Sanyal A, Gerassimova TI, Lajoie BR, Bell JS, et al. Architectural protein subclasses shape 3D organization of genomes during lineage commitment. Cell. 2013;153(6):1281-95.
45. Zhang Y, Wong CH, Birnbaum RY, Li G, Favaro R, Ngar CY, et al. Chromatin connectivity maps reveal dynamic promoter-enhancer long-range associations. Nature. 2013;504(7479):306-10.
46. Lupiánez DG, Spielmann M, Mundlos S. Breaking TADs: How Alterations of Chromatin Domains Result in Disease. Trends Genet. 2016;32(4):225-37.
47. Schoenfelder S, Furlan-Magaril M, Mifsud B, Tavares-Cadete F, Sugar R, Javierre BM, et al. The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. Genome Res. 2015;25(4):582-97.
48. Symmons O, Uslu VV, Tsuji mur T, Rufs S, Narsari S, Schwarz W, et al. Functional and topological characteristics of mammalian regulatory domains. Genome Res. 2014;24(3):390-400.
49. Entrenval M, Schuettengruber B, Cavalli G. Regulation of Genome Architecture and Function by Polycomb Proteins. Trends Cell Biol. 2016;26(7):511-25.
50. Lee HG, Kahn TG, Simcox A, Schwartz YB, Pirrotta V. Genome-wide activities of Polycomb complexes control pervasive transcription. Genome Res. 2015;25(8):1170-81.
51. Luo X, Dangaria S, Ito Y, Walker CG, Jin T, Schmidt MK, et al. Neural crest lineage segregation: a blueprint for periodontal regeneration. J Dent Res. 2009;88(9):781-91.
52. Gopinathan G, Kolokythas A, Luan X, Diekwisch TG. Epigenetic marks define the lineage and differentiation potential of two distinct neural crest-derived intermediate odontogenic progenitor populations. Stem Cells Dev. 2013;22(12):1763-78.
53. Karaoz E, Dogan BN, Aksoy A, Gacar G, Ak yüz S, Ayhan A, et al. Isolation and in vitro characterisation of dental pulp stem cells from natal teeth. Histochem Cell Biol. 2010;133(1):95-112.
54. da Cunha JM, da Costa-Neves A, Kerkis I, da Silva MC. Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons in vitro; implications for tissue engineering and repair in the nervous system. Eur J Neurosci. 2004;19(9):2385-98.
55. da Cunha JM, da Costa-Neves A, Kerkis I, da Silva MC. Deregulation and therapeutic potential of microRNAs in arthritic diseases. Nat Rev Rheumatol. 2016;12(4):211-20.
56. Phillips-Cremins JE, Sauria ME, Sanyal A, Gerassimova TI, Lajoie BR, Bell JS, et al. Architectural protein subclasses shape 3D organization of genomes during lineage commitment. Cell. 2013;153(6):1281-95.
tent Stem Cell Potential by Simultaneously Initiating and Repressing Enhancer Activity. Cell Stem Cell. 2016;18(1):104-17.
67. Fujita K, Ogawa R, Ito K, CHD7, Oct3/4, Sox2, and Nanog control FoxD3 expression during mouse neural crest-derived stem cell formation. FEBS J. 2016; in press.
68. Harikumar A, Meshorer E. Chromatin remodeling and bivalent histone modifications in embryonic stem cells. EMBO Rep. 2015;16(12):1609-19.
69. Tee WW, Reinberg D. Chromatin features and the epigenetic regulation of pluripotency states in ESCs. Development. 2014;141(12):2376-90.
70. Smith ZD, Sindhu C, Meissner A. Molecular features of cellular reprogramming and development. Nat Rev Mol Cell Biol. 2016;17(3):139-54.
71. Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov. 2014;13(9):673-91.
72. Galvani A, Thiriet C. Nucleosome Dancing at the Tempo of Histone Tail Acetylation. Genes (Basel). 2015;6(3):607-21.
73. Creighton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A. 2010;107(50):21931-6.
74. Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J. A unique chromatin signature uncovers early developmental enhancers in humans. Nature. 2011;470(7333):279-83.
75. Hezroni H, Tzchori I, Davidi A, Mattout A, Biran A, Nissim-Rafinia M, et al. H3K9 histone acetylation predicts pluripotency and reprogramming capacity of ES cells. Nucleus. 2011;2(4):300-9.
76. Zentner GE, Tesar PJ, Saccheri PC. Epigenetic signatures distinguish multiple classes of enhancers with distinct cellular functions. Genome Res. 2011;21(8):1273-83.
77. Karmodiya K, Krebs AR, Oulad-Abdelghani M, Kimura H, Tora L. H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. BMC Genomics. 2012;13:424.
78. Paradowska AS, Miller D, Spiess AN, Vieweg M, Cerna M, Dvorakova-Hortova K, et al. Genome wide identification of promoter binding sites for H4K12ac in human sperm and its relevance for early embryonic development. Epigenetics. 2012;7(9):1057-70.
79. Troupner P, Pott S, Keller C, Kamieniarz-Gidula K, Caron M, Richter F, et al. Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer. Cell. 2013;152(4):859-72.
80. Taylor GC, Eskeland R, Hekimoglu-Balkan B, Pradeepa MM, Bickmore WA. H4K16 acetylation marks active genes and enhancers of embryonic stem cells, but does not alter chromatin compaction. Genome Res. 2013;23(12):2053-65.
81. Kumar V, Rayan NA, Muratani M, Linn S, Elangovan B, Lixia X, et al. Comprehensive benchmarking reveals H2BK20 acetylation as a distinctive signature of cell-state-specific enhancers and promoters. Genome Res. 2016;26(5):812-23.
82. Das C, Lucia MS, Hansen KC, Tyler JK. CBP/p300-mediated acetylation of histone H3 on lysine 56. Nature. 2009;459(7243):113-7.
83. Stejskal S, Tesařová L, Koutná I. Mysterious role of H3K56ac in embryonic stem cells. Folia Biol (Praha). 2014;60 Suppl 1:71-5.
84. Pradeepa MM, Grimes GR, Kumar Y, Olley G, Taylor GC, Schneider R, et al. Histone H3 globular domain acetylation identifies a new class of enhancers. Nat Genet. 2016;48(6):681-86.
85. Bhanu NV, Siddi S, Garcia BA. Histone modification profile reveals differential signatures associated with human embryonic stem cell self-renewal and differentiation. Proteomics. 2016;16(3):448-58.
86. Lalonde ME, Cheng X, Côté J. Histone target selection within chromatin: an exemplary case of teamwork. Genes Dev. 2014;28(10):1029-41.
87. Yang XJ. MOZ and MORF acetyltransferases: Molecular interaction, animal development and human disease. Biochim Biophys Acta. 2015;1853(8):1818-26.
88. Dancy BM, Cole PA. Protein lysine acetylation by p300/CBP. Chem Rev. 2015;115(6):2419-52.
89. Ring A, Kim YM, Kahn M. Wnt/catenin signaling in adult stem cell physiology and disease. Stem Cell Rev. 2014;10(4):1252.
90. Skalska L, Stojnic R, Li J, Fischer B, Cerda-Moya G, Sakai H, et al. Chromatin signatures at Notch-regulated enhancers reveal large-scale changes in H3K36ac upon activation. EMBO J. 2015;34(14):1889-904.
91. Tan Y, Xue Y, Song C, Grunstein M. Acetylated histone H3K56 interacts with Oct4 to promote mouse embryonic stem cell pluripotency. Proc Natl Acad Sci U S A. 2013;110(28):11493-8.
92. Bedford DC, Brindle PK. Is histone acetylation the most important physiological function for CBP and p300? Aging. 2012;4(4):247-255.
93. Kim MS, Cho HI, Park SH, Kim JH, Chai YG, Jang YK. The histone acetyltransferase Mst2 regulates Nanog expression, and is involved in maintaining pluripotency and self-renewal of embryonic stem cells. FEBS Lett. 2015;589(8):941-50.
94. Kueh AJ, Dixon MP, Voss AK, Thomas T. HBO1 is required for H3K14 acetylation and normal transcriptional activity during embryonic development. Mol Cell Biol. 2013;31(4):845-60.
95. Li X, Li L, Pandey R, Byun JS, Gardner K, Qin Z, et al. The histone acetyltransferase MOF is a key regulator of the embryonic stem cell core transcriptional network. Cell Stem Cell. 2012;11(2):163-78.
96. Ravens S, Fourmier M, Ye T, Sterle M, Dembele D, Chavant V, et al. Mof-associated complexes have overlapping and unique roles in regulating pluripotency in embryonic stem cells and during differentiation. Elife. 2014;3.
97. Sheikh BN, Downer NL, Pipson B, Vanyai HK, Kueh AJ, McCarthy DJ, et al. MOZ and BMW1 play opposing roles during Hox gene activation in ES cells and in body segment identity specification in vivo. Proc Natl Acad Sci U S A. 2012;110(9):3437-42.
98. Dreveny I, Deevse SE, Fulton J, Yue B, Messmer M, Bhat-Chandraya A, et al. The double PHD finger domain of MOZ/MYST3 induces α-helical structure of the histone H3 tail to facilitate acetylation and methylation sampling and modification. Nucleic Acids Res. 2014;42(2):825-5.
99. Wang L, Dent SY. Functions of SAGA in development and disease. Epigenomics. 2014;6(3):329-39.
100. Hirsch CL, Coban Akdemir Z, Wang L, Jayakumar G, Trcka D, Weiss A, et al. Myc and SAGA rewire an alternative splicing network during early somatic cell reprogramming. Genes Dev. 2015;29(8):803-16.
101. Kaimori JY, Machara K, Hayashi-Takanaka Y, Harada A, Fukuda M, Yamamoto S, et al. Histone H4 lysine 20 acetylation is associated with gene repression in human cells. Sci Rep. 2016;6:24318.
102. Laugesen A, Helin K. Chromatin repressive complexes in stem cells, development, and cancer. Cell Stem Cell. 2014;14(6):735-51.
103. Meier K, Brehm A. Chromatin regulation: how complex does it get? Epigenetics. 2014;9(11):1485-95.
104. Dovey OM, Foster CT, Cowley SM. Histone deacetylase 1 (HDAC1), but not HDAC2, controls embryonic stem cell differentiation. Proc Natl Acad Sci U S A. 2010;107(18):8242-7.
105. Hoxha E, Lambers E, Xie H, De Andrade A, Krishnamurthy P, Wasserstrom JA, et al. Histone deacetylase 1 de-
ficiency impairs differentiation and electrophysiological properties of cardiomyocytes derived from induced pluripotent cells. Stem Cells. 2012;30(11):2412-22.

106. Aranda S, Rutishauser D, Ernfors P. Identification of a large protein network involved in epigenetic transmission in replicating DNA of embryonic stem cells. Nucleic Acids Res. 2014;42(11):6972-86.

107. Kidder BL, Palmer S. HDAC1 regulates pluripotency and lineage specific transcriptional networks in embryonic and trophoblast stem cells. Nucleic Acids Res. 2012;40(7):2925-38.

108. Jamaaladdin S, Kelly RD, O’Regan L, Dovey OM, Hudson GE, Millard CJ, Portolano N, et al. Histone deacetylase (HDAC) 1 and 2 are essential for accurate cell division and the pluripotency of embryonic stem cells. Proc Natl Acad Sci U S A. 2014;111(27):9840-5.

109. Kadamb R, Mittal S, Bansal N, Batra H, Saluja D. Sin3: in etchegaray JP, Chavez L, Huang Y, Ross KN, Choi J, MarGeisler SJ, Paro R. Trithorax and Polycomb group-dependent H2A mono-ubiquitination is a crucial step to mediate PRC1-dependent repression of developmental genes to maintain ES cell identity. PLoS Genet. 2012;8(7):e1002774.

110. Porcheron D, Broussolle E, Goff S, et al. Targeting polycomb to pericentric heterochromatin in embryonic stem cells reveals a role for HDAC1 in PRC2 recruitment. Cell Rep. 2014;7(5):1456-70.

111. Hu G, Wade PA. NuRD and pluripotency: a complex balancing act. Stem Cell Biol. 2013;92(8-9):237-46.

112. Reynolds N, Salmon-Divon M, Dovey OM, Hudson GE, Millard CJ, Portolano N, et al. Histone deacetylase (HDAC) 1 and 2 are essential for accurate cell division and the pluripotency of embryonic stem cells. Proc Natl Acad Sci U S A. 2014;111(27):9840-5.

113. Bosch-Presegú E, Vaquero A. Sirtuin-dependent epigenetic maintenance in the regulation of developmental genes. FEBS J. 2015;282(9):1745-67.

114. Kuzmichev A, Margueron R, vaquero A, Preissner TS, He. SIRT1 positively regulates autophagy and mitochondria localization. Cell Stem Cell. 2008;2(3):241-51.

115. Ou Y, Zhang J, Wu S, Li B, Liu S, Cheng J. SIRT1 promotes proliferation and inhibits apoptosis of human malignant glioma cell lines. Neurosci Lett. 2012;525(2):168-72.

116. Han MK, Song EK, Guo Y, Ou X, Mantel C, Broxmeyer HE. SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. Cell Stem Cell. 2008;2(3):241-51.

117. Calvanese V, Lara e, Suárez-Alvarez B, Abu Dawud R, Vázquez-Chantada M, Martínez-Chantar ML, et al. Sirtuin 1 regulates of developmental genes during differentiation of stem cells. Proc Natl Acad Sci U S A. 2010;107(31):13736-41.

118. Chae HD, Broxmeyer HE. SIRT1 deficiency downregulates Pten/Jnk/Fox01 pathway to block reactive oxygen species-induced apoptosis in mouse embryonic stem cells. Stem Cells Dev. 2011;20(7):1277-85.

119. Ou X, Lee MR, Huang X, Messina-Graham S, Broxmeyer HE. SIRT1 positively regulates autophagy and mitochondria function in embryonic stem cells under oxidative stress. Stem Cells. 2014;32(5):1183-94.

120. Etchegaray JP, Chavez L, Huang Y, Ross KN, Choi J, Martinez-Pastor B, et al. The histone deacetylase SIRT6 controls embryonic stem cell fate via TET-mediated production of 5-hydroxymethylcytosine. Nat Cell Biol. 2015;17(5):545-57.

121. Geisler SJ, Paro R. Trithorax and Polycold group-dependent regulation: a tale of opposing activities. Development. 2015;142(7):2876-87.

122. Zhang T, Cooper S, Brockdorff N. The interplay of histone modifications - writers that read. EMBO Rep. 2015;16(11):1467-81.

123. Vázquez-Chantada M, Martínez-Chantar ML, et al. Sirtuin 1 mediates LINe elements in mouse embryonic stem cells. Mol Cell. 2014;55(2):277-90.

124. Piunti A, Shilatifard A. Epigenetic balance of gene expression by Polycomb and COMPASS families. Science. 2016;352(6290):aa9780.

125. Jadhav U, Nalapareddy K, Saxena M, O'Neill NK, Pinello L, Yuan GC, et al. Acquired Tissue-Specific Promoter Bispeclicity Is a Basis for PRC2 Necessity in Adult Cells. Cell. 2016;165(6):1389-400.

126. Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisi F, et al. PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. Mol Cell. 2012;45(3):344-56.

127. Endoh M, Endoh T, Isokohji N, Ohara O, Kondo T, King Hw, McBurney M, Müller C, Vermeulen M, Müller J. Histone H2A mono-ubiquitination promotes histone H3 methylation in Polyclomb repression. Nature. 2014;516(7531):569-71.

128. Blackledge NP, Farcas AM, Kondo T, King HW, McBurney M, Hanssen LL, et al. Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. Cell. 2014;157(6):1445-59.

129. Buhtz-Karsioglu A, De La Rosa-velázquez IA, Ramirez F, McBurney M, Rose NR, et al. KDM2B links the Polycomb Repressive Complex 1 (PRC1) to recognition of CpG islands. Elife. 2012;1:e00205.

130. Calvanese V, Lara e, Suárez-Alvarez B, Abu Dawud R, Vázquez-Chantada M, Martínez-Chantar ML, et al. Sirtuin 1 regulation of developmental genes during differentiation of stem cells. Proc Natl Acad Sci U S A. 2010;107(31):13736-41.

131. Xue J, Kim H, Huang R, Lu W, Tang M, Shi F, et al. The Daxx/Atax Complex Protects Tandem Repetitive Elements during DNA Hypomethylation by Promoting H3K9 Methylation. Cell Stem Cell. 2015;17(3):273-86.

132. Shinkai Y, Tachibana M. H3K9 methyltransferase G9a and the related molecule GLP. Genes Dev. 2011;25(8):781-94.

133. Barenboim M, Onishi-Seebacher M, Arand J, et al. Suv39h1-dependent H3K9me3 marks intact retrotransposons and silences LINE elements in mouse embryonic stem cells. Mol Cell. 2014;55(2):277-90.

134. Maeda I, Okamura D, Tokitake Y, Ikeda M, Kawaguchi H, Mise N, et al. Max is a repressor of germ cell-related gene expression in mouse embryonic stem cells. Nat Commun. 2013;4:1754.

135. Shinkai Y, Tachibana M. H3K9 methyltransferase G9a and the related molecule GLP. Genes Dev. 2011;25(8):781-94.

136. Brinkman B, Brouwers M, Oomen T, et al. LSH and G9a/GLP complex are required for developmentally distinct PRC1 family complexes. Mol Cell. 2014;55(5):545-57.

137. Shinkai Y, Tachibana M. H3K9 methyltransferase G9a and the related molecule GLP. Genes Dev. 2011;25(8):781-94.

138. Blackledge NP, Farcas AM, Kondo T, King HW, McBurney M, Hanssen LL, et al. Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. Cell. 2014;157(6):1445-59.

139. Pan MR, Hsu MC, Chen LT, Hung wC. G9a orchestrates cell cycle progression by recruiting Polycomb Repressive Complex 2 and KDM7A to promote histone H3K27 methylation. Cell Rep. 2015;2(3):593-605.

140. Zylicz JJ, Dietmann S, Günesdogan U, Hackett JA, Cougot D, Lee C, et al. Chromatin dynamics and the role of G9a in gene regulation and enhancer silencing during early mouse development. Elife. 2015;4.

141. Piunti A, Shilatifard A. Epigenetic balance of gene expression by Polycomb and COMPASS families. Science. 2016;352(6290):aa9780.

142. Pan MR, Hsu MC, Chen LT, Hung WC, G9a orchestrates PCL3 and KDM7A to promote histone H3K27 methylation. Sci Rep. 2015;5:18709.
murine embryonic stem cell differentiation toward endo-
card. Cell Rep. 2014;8(6):1989-2002.

143. Ohhata T, Matsumoto M, Leeb M, Shibata S, Sakai S, Kita-
gawa K, et al. Histone H3 Lysine 36 Trimethylation Is Es-
tablished over the Xist Promoter by Antisense Tiss
Transcription and Contributes to Repressing Xist Expre-
sion. Mol Cell Biol. 2015;35(22):3909-20.

144. Castaño J, Moreira C, Sesé B, Boue S, Bonet-Costa C, Martí
M, et al. SETDB7 Regulates the Differentiation of Human
Embryonic Stem Cells. PLoS One. 2016;11(2):e0149502.

145. Benayoun BA, Pollina EA, Ucar D, Mahmoudi S, Karra K,
Wong ED, et al. H3K4me3 breadth is linked to cell identity
and transcriptional consistency. Cell. 2014;158(3):673-88.

146. Ucar D, Bayarsaihan D. Cell-specific gene promoters are
marked by broader spans of H3K4me3 and are associated
with robust gene expression patterns. Epigenomics. 2015;7(2):129-31.

147. Shilatifard A. The COMPASS family of histone H3K4
methylases: mechanisms of regulation in development and
disease pathogenesis. Annu Rev Biochem. 2012;81:65-95.

148. Wu M, Wang PF, Lee JS, Martin-Brown S, Florens L,
Washburn M, et al. Molecular regulation of H3K4 trimeth-
lyation by Wdr82, a component of human Set1/COMPASS.
Mol Cell Biol. 2008;28(24):7337-44.

149. Schuettengruber B, Martinez AM, Iovino N, Cavalli G.
Trithorax group proteins: switching genes on and keeping
them active. Nat Rev Mol Cell Biol. 2011;12(12):799-814.

150. Shinsky SA, Montetith KE, Viggiano S, Cosgrove MS.
Biochemical reconstitution and phylogenetic comparison of
human SET1 family core complexes involved in histone
methylation. J Biol Chem. 2013;289(10):6361-75.

151. Ladopoulos V, Hofemeister H, Hoogenkamp M, Riggs
AD, Stewart AF, Bonifer C. The histone methyltransferase
KMT2B is required for RNA polymerase II association and
protection from DNA methylation at the MagohB CpG island
promoter. Mol Cell Biol. 2013;3(3):1383-93.

152. Herz HM, Mohan M, Garrass AS, Liang K, Takahashi YH,
Mickey K, et al. Enhancer-associated H3K4 monomethyla-
ytion by Trithorax-related, the Drosophila homolog of mam-
malian MLL3/ML14. Genes Dev. 2012;26(23):2504-20.

153. Hu D, Gao X, Morgan MA, Herz HM, Smith ER, Shilati-
fid A. The MLL3/ML14 branches of the COMPASS fam-
ily function as major histone H3K4 monomethylases at
enhancers. Mol Cell Biol. 2013;33(23):4745-54.

154. Cheng J, Blum R, Bowman C, Hu D, Shilatifard A, Shen
S, et al. A role for H3K4 monomethylation in gene repression
and partitioning of chromatin readers. Mol Cell. 2014;53(6):979-92.

155. Clouaire T, Webb S, Bird A. Cfp1 is required for gene ex-
pression-dependent H3K4 trimethylation and H3K9 acety-
lation in embryonic stem cells. Genome Biol. 2014;15(9):451.

156. Matsumura Y, Nakaki R, Inagaki T, Yoshida A, Kano Y,
Kimura H, et al. H3K4/H3K9me3 Bivalent Chromatin Do-
 mains Targeted by Lineage-Specific DNA Methylation
Pauses Adipocyte Differentiation. Mol Cell. 2015;60(4):584-
96.

157. Adamo A, Sesé B, Boue S, Castaño J, Paramonov I, Bar-
rero MJ, et al. LSD1 regulates the balance between self-
renewal and differentiation in human embryonic stem cells.
Nat Cell Biol. 2011;13(6):652-9.

158. Foster CT, Dovey OM, Lalezin L, Luo JL, Gant TW, Bar-
lev N, et al. Lysine-specific demethylase 1 regulates the em-
byronic transcriptional and CoREST stability. Mol Cell Biol.
2010;30(20):4851-63.

159. Yin F, Lan R, Zhang X, Zhu L, Chen F, Xu Z, et al. LSD1
regulates pluripotency of embryonic stem/carcinoma cells
through histone deacetylase 1-mediated deacetylation of
histone H4 at lysine 16. Mol Cell Biol. 2014;34(2):158-79.

160. Fang R, Barbera AJ, Xu Y, Rutenberg M, Leonor T, Bi Q,
et al. Human LSD2/KDM1b/AOF1 regulates gene tran-
scription by modulating intragenic H3K4me2 methylation.
Mol Cell. 2010;39(2):222-33.

161. He J, Shen L, Wan M, Taranova O, Wu H, Zhang Y.
Kdm2b maintains murine embryonic stem cell status by re-
cruiting PRC1 complex to CpG islands of developmental
genes. Nat Cell Biol. 2013;15(4):373-84.

162. Boulard M, Edwards JR, Bestor TH. FBXL10 protects
Polycomb-bound genes from hypermethylation. Nat Genet.
2015;47(5):479-85.

163. Pedersen MT, Kooststra SM, Radziszewska A, Laugesen
A, Johansen JV, Hayward DG, et al. Continual removal of
H3K9 promoter methylation by Mjmd2 demethylases is vital
for ESC self-renewal and early development. EMBO J.
2016;35(14):1550-64.

164. Beshiri ML, Holmes KB, Richter WF, Hess S, Islam AB,
Yan Q, et al. Coordinated repression of cell cycle genes by
KDM5A and E2F4 during differentiation. Proc Natl Acad
Sci U S A. 2012;109(45):18499-504.

165. Kidder BL, Hu G, Zhao K. KDM5B focuses H3K4 methy-
lization near promoters and enhancers during embryonic stem
cell self-renewal and differentiation. Genome Biol.
2014;15(2):R32.

166. Xie L, Pelz C, Wang W, Bashar A, Varlamova O, Shadle
S, et al. KDM5B regulates embryonic stem cell self-renewal
and represses cryptic intragenic transcription. EMBO J.
2011;30(8):1473-84.

167. Outchkourov NS, Munio JM, Kaufmann K, van Vlcken WF,
Groot Koerkamp MJ, van Leenen D, et al. Balancing of his-
tone H3K4 methylation states by the Kdm5c/SMCX histo-
tone demethylase modulates promoter and enhancer
function. Cell Rep. 2013;3(4):1071-9.

168. Welstead GG, Creyghton MP, Bilodeau S, Cheng AW,
Markoulaki S, Young RA, et al. X-linked H3K27me3
demethylase Utx is required for embryonic development in
a sex-specific manner. Proc Natl Acad Sci U S A.
2012;109(32):13004-9.

169. Morales Torres C, Laugesen A, Helin K. Utx is required for
proper induction of ectoderm and mesoderm during differ-
entiation of embryonic stem cells. PLoS One. 2013;8(4):e60020.

170. Ohtani K, Zhao C, Dobreva G, Manavski Y, Kluge B,
Braun T, et al. Jmd3 controls mesodermal and cardiovascu-
lar differentiation of embryonic stem cells. Circ Res.
2013;113(7):856-62.

171. Tang Y, Hong YZ, Bai HJ, Wu Q, Chen CD, Lang JY, et
al. PHF8 regulates mesodermal and cardiac differentiation
in embryonic stem cells through mediating the histone
demethylation of pmaip1. Stem Cells. 2016;34(6):1527-40.

172. Bowman EA, Kelly WG. RNA polymerase II transcription
elongation and Pol II CTD Ser2 phosphorylation: A tail of
two kinases. Nucleus. 2014;5(3):224-36.

173. Jasnovidova O, Stefl R. The CTD code of RNA polymerase
II: a structural view. Wiley Interdiscip Rev RNA.
2013;4(1):1-16.

174. Stock JK, Giadrossi S, Casanova M, Brookes E, Vidal M,
Koseki H, Brockdorff N, et al. Ring1-mediated ubiquitina-
tion of H2A restrains poised RNA polymerase II at bivalent
genes in mouse ES cells. Nat Cell Biol. 2007;9(12):1428-35.

175. Markoulaki S, Young RA, et al. X-linked H3K27me3
demethylase Utx is required for embryonic development in
a sex-specific manner. Proc Natl Acad Sci U S A.
2012;109(32):13004-9.

176. Boulard M, Edwards JR, Bestor TH. FBXL10 protects
Polycomb-bound genes from hypermethylation. Nat Genet.
2015;47(5):479-85.

177. Beshiri ML, Holmes KB, Richter WF, Hess S, Islam AB,
Yan Q, et al. Coordinated repression of cell cycle genes by
KDM5A and E2F4 during differentiation. Proc Natl Acad
Sci U S A. 2012;109(45):18499-504.

178. Kidder BL, Hu G, Zhao K. KDM5B focuses H3K4 methy-
lization near promoters and enhancers during embryonic stem
cell self-renewal and differentiation. Genome Biol.
2014;15(2):R32.
utination regulatory pathway is required for differentiation of multipotent stem cells. Mol Cell. 2012;46(5):705-713.

179. Yang W, Lee YH, Jones AE, Woolnough JL, Zhou D, Dai Q, Wu Q, et al. The histone H2A deubiquitinase Usp16 regulates embryonic stem cell gene expression and lineage commitment. Nat Commun. 2014;5:3818.

180. Marmorstein R, Zhou MM. Writers and readers of histone acetylation: structure, mechanism, and inhibition. Cold Spring Harb Perspect Biol. 2014;6(7):a018762.

181. Whyte WA, Orlando DA, Hnizd D, Abraham BJ, Lin CY, Kagey MH, et al. Master transcription factors and mediators establish super-enhancers at key cell identity genes. Cell. 2013;153(2):307-19.

182. Niederriter AR, Varshney A, Parker SC, Martin DM. Super Enhancers in Cancers, Complex Disease, and Developmental Disorders. Genes (Basel). 2015;6(4):1183-200.

183. Pott S, Lieb JD. What are super-enhancers? Nat Genet. 2015;47(1):8-12.

184. Devalia BN, Case-Borden C, Gegonne A, Hsu CH, Chen Q, Meerzaman D, et al. BRD4 is a histone acetyltransferase that evicts nucleosomes from chromatin. Nat Struct Mol Biol. 2016;23(6):540-8.

185. Escamilla-Del-Arenal M, da Rocha ST, Spruijt CG, Masui N, Niederriter AR, varshney A, Parker SC, Martin DM. Super Enhancers in Cancers, Complex Disease, and Developmental Disorders. Genes (Basel). 2015;6(4):1183-200.

186. Lin JJ, Lehmann LW, Bonora G, Sridharan R, vashisht P, Lieb JD. What are super-enhancers? Nat Genet. 2015;47(1):8-12.

187. Schnetz MP, Handoko L, Akhtar-Zaidi B, Bartels CF, Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and function of mammalian DNA methyltransferases. Chembiochem. 2011;12(2):206-22.

188. Dealton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011;5(10):1010-22.

189. Vavouri T, Lehner B. Human genes with CpG island promoters have a distinct transcription-associated chromatin organization. Genome Biol. 2012;13(11):R110.

190. Mantsoki A, Devailly G, Joshi A. CpG island erosion, polycomb occupancy and sequence motif enrichment at bivalent promoters in mammalian embryonic stem cells. Sci Rep. 2015;5:16791.

191. Liyanage VR, Jarmasz JS, Murugeshan N, Del Bigio MR, Athanasiadou R, de Sousa D, Myant K, Merusi C, Reddington JP, Perricone SM, Nestor CE, Reichmann J, Youngson NA, Suzuki M, et al. Redistribution of H3K27me3 upon DNA hypomethylation results in de-recruitment of Polycomb target genes. Genome Biol. 2013;14(3):R25.

192. Liao J, Karnik R, Gu H, Ziller MJ, Clement K, Tsanov AM, et al. Targeted disruption of DNMT1, DNMT3A and DNMT3B in human embryonic stem cells. Nat Genet. 2015;47(5):469-78.

193. Petell CJ, Alabdi L, He M, San Miguel P, Rose R, Gowher H. An epigenetic switch regulates de novo DNA methyla-

194. Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011;5(10):1010-22.

195. Kang J, Lienhard M, Pastor WA, Chawla A, Novotny M, Tsagaratou A, et al. Simultaneous deletion of the methylcytosine oxidases Tet1 and Tet3 increases transcriptome variability in early embryogenesis. Proc Natl Acad Sci U S A. 2015;112(31):E4236-45.

196. Dawlaty MM, Breiling A, Le T, Barrasa MI, Raddatz G, Gao Q, et al. Loss of Tet enzymes compromises proper differentiation of embryonic stem cells. Dev Cell. 2014;29(1):102-11.

197. Zhang Q, Zhao K, Shen Q, Han Y, Gu Y, Li X, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress Il-6. Nature. 2015;525(7569):389-93.

198. Perera A, Eisen D, Wagner M, Laube SK, Künzel AF, Koch S, et al. TET3 is recruited by REST for context-specific hydroxymethylation and induction of gene expression. Cell Rep. 2015;11(2):283-94.

199. Zeng Y, Yao B, Jin J, Lin M, Kim N, Song Q, et al. Lin28A Binds Active Promoters and Recruits Tet1 to Regulate Gene Expression. Mol Cell. 2016;61(1):153-60.

200. Bohan CP, Bunting S, Choudhury S, Enver T, Karin M, SHEARER JK, et al. MBD2 and MBD3: elusive functions and mechanisms. Front Genet. 2014;5:428.

201. Varshney A, Parker SC, Martin DM. Super Enhancers in Cancers, Complex Disease, and Developmental Disorders. Genes (Basel). 2015;6(4):1183-200.

202. Sun W, Guan M, Li X. 5-hydroxymethylcytosine-mediated DNA demethylation in stem cells and development. Stem Cells Dev. 2014;23(9):923-30.

203. Rasmussen KD, Helin K. Role of TET enzymes in DNA methylation, development, and cancer. Genes Dev. 2016;30(7):733-50.

204. Kang J, Lienhard M, Pastor WA, Chawla A, Novotny M, Tsagaratou A, et al. Simultaneous deletion of the methylcytosine oxidases Tet1 and Tet3 increases transcriptome variability in early embryogenesis. Proc Natl Acad Sci U S A. 2015;112(31):E4236-45.

205. Bohan CP, Bunting S, Choudhury S, Enver T, Karin M, SHEARER JK, et al. MBD2 and MBD3: elusive functions and mechanisms. Front Genet. 2014;5:428.

206. Zhang Q, Zhao K, Shen Q, Han Y, Gu Y, Li X, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress Il-6. Nature. 2015;525(7569):389-93.

207. Perera A, Eisen D, Wagner M, Laube SK, Künzel AF, Koch S, et al. TET3 is recruited by REST for context-specific hydroxymethylation and induction of gene expression. Cell Rep. 2015;11(2):283-94.

208. Zeng Y, Yao B, Jin J, Lin M, Kim N, Song Q, et al. Lin28A Binds Active Promoters and Recruits Tet1 to Regulate Gene Expression. Mol Cell. 2016;61(1):153-60.
259. Nayak A, Viale-Bouroncle S, Morsczech C, Muller S. The SUMO-specific isopeptidase SENP3 regulates ML1.1/ML2 methyltransferase complexes and controls osteogenic differentiation. Mol Cell. 2014;55(1):47-58.

260. Hoang M, Kim JH, Kim Y, Tong E, Trammell B, Liu Y, et al. Alcohol-induced suppression of KDM6B dysregulates the mineralization potential in dental pulp stem cells. Stem Cell Res. 2016;17(1):111-121.

261. Hassler MR, Kliarasoka A, Kollmann K, Steiner I, Bilban M, Schiefer AI, et al. Antineoplastic activity of the DNA methyltransferase inhibitor 5-aza-2‘-deoxycytidine in anaplastic large cell lymphoma. Biochimie. 2012;94(11):2297-307.

262. Cardoso FP, de Faria Amormino SA, Dutra WO, Ribeiro Sobrinho AP, Moreira PR. Methylation pattern of the CD14 and TLR2 genes in human dental pulp. J Endod. 2014;40(3):384-6.

263. Zhang D, Li Q, Rao L, Yi B, Xu Q. Effect of 5-Aza-2'-deoxycytidine on Odontogenic Differentiation of Human Dental Pulp Cells. J Endod. 2015;41(5):640-5.

264. Nakatsuka R, Nozaki T, Uemura Y, Matsuka Y, Sasaki Y, Shimohara M, et al. 5-Aza-2'-deoxycytidine treatment induces skeletal myogenic differentiation of mouse dental pulp stem cells. Arch Oral Biol. 2010;55(5):350-7.

265. Yoshioka H, Minamizaki T, Yoshiko Y. The dynamics of DNA methylation and hydroxymethylation during amelogenesis. Histochem Cell Biol. 2015;144(5):471-8.

266. Rao LJ, Yi BC, Li QM, Xu Q. TET1 knockdown inhibits the odontogenic differentiation potential of human dental pulp cells. Int J Oral Sci. 2016;8(2):110-6.

267. Singh RP, Massachi I, Manickavel S, Singh S, Rao NP, Hasan S, et al. The role of miRNA in inflammation and autoimmunity. Autoimmun Rev. 2013;12:1160-65.

268. Elling R, Chan J, Fitzgerald KA. Emerging role of long noncoding RNAs as regulators of innate immune cell development and inflammatory gene expression. Eur J Immunol. 2016;46(5):504-12.

269. Marques-Rocha JL, Sambas M, Milagro FI, Bressan J, Sevigny R, Chan J, Fitzgerald KA. Emerging role of long noncoding microRNAs’ patterns in human dental pulp stem cells. J Cell Mol Med. 2015;19(3):566-80.

270. Liu W, Gong Q, Ling J, Zhang W, Liu Z, Quan J. Role of miR-424 on angiogenic potential in human dental pulp cells. J Endod. 2014;40(1):76-82.

271. Gu S, Ran S, Liu B, Liang J. miR-152 induces human dental pulp stem cell senescence by inhibiting SIRT7 expression. FEBS Lett. 2016;590(8):1123-31.

272. Chen L, Song Z, Huang S, Wang R, Qin W, Gao J, et al. IncRNA DANCR suppresses odontoblast-like differentiation of human dental pulp cells by inhibiting Wnt/β-catenin pathway. Cell Tissue Res. 2016;364(2):309-18.

273. Hu D, Young NM, Xu Q, Jimmichzy H, Green RM, Mio W, et al. Signals from the brain induce variation in avian facial shape. Dev Dyn. 2015; in press.

274. Emera D, Yin J, Reilly SK, Gockley J, Noonan JP. Origin and evolution of developmental enhancers in the mammalian neocortex. Proc Natl Acad Sci U S A. 2016;113(19):E2617-26.

275. Marcucio R, Hallgrimson B, Young NM. Facial Morphogenesis: Physical and Molecular Interactions Between the Brain and the Face. Curr Top Dev Biol. 2015;115:299-320.

276. Martinez-Ja, Marti A. Noncoding RNAs, cytokines, and inflammation-related diseases. FASEB J. 2015;29(9):3595-511.

277. Zhong S, Zhang S, Bair E, Nares S, Khan AA. Differential expression of microRNAs in normal and inflamed human pulp. J Endod. 2012;38(6):746-52.

278. Gong T, Heng BC, Lo EC, Zhang C. Current Advance and Future Prospects of Tissue Engineering Approach to Dentin/Pulp Regenerative Therapy. Stem Cells Int. 2016;2016:9204574.

279. Gay I, Cavender A, Peto D, Sun Z, Speer A, Cao H, et al. Differentiation of human dental stem cells reveals a role for microRNA-218-1 in microRNA signals in human dental pulp cells with inflammatory response. J Endod. 2014;40(1):101-8.

280. Huang X, Xu S, Gao J, Liu F, Yue J, Chen T, et al. miRNA expression profiling identifies DSP5 regulators in cultured dental pulp cells. Int J Mol Med. 2011;28(4):659-67.

281. Yue J, Wu B, Gao J, Huang X, Li C, Ma D, et al. DMP1 is a target of let-7 in dental pulp cells. Int J Mol Med. 2012;30(2):295-301.

282. Liu H, Lin H, Zhang L, Sun Q, Yuan G, Zhang L, et al. miR-145 and miR-143 regulate odontoblast differentiation through targeting Ki67 and Osx genes in a feedback loop. J Biol Chem. 2013;288(13):9261-71.

283. Li D, Deng T, Li H, Li Y. MiR-143 and miR-135 inhibitors treatment induces skeletal myogenic differentiation of human adult dental pulp stem cells. Arch Oral Biol. 2015;60(11):1613-7.

284. Hara ES, Ono M, Eguchi T, Kubota S, Pham HT, Sonoyama W, et al. miRNA-720 controls stem cell phenotype, proliferation and differentiation of human dental pulp cells. PLoS One. 2013;8(12):e83545.

285. Wang K, Li L, Wu J, Qiu Z, Zhou F, Wu H. The different expression profiles of microRNAs in elderly and young human dental pulp and the role of miR-433 in human dental pulp cells. Mech Ageing Dev. 2015;146-148C:1-11.

286. Vasanthan P, Govindasamy V, Gnanasegaran N, Kunasekaran W, Masa S, Abu Kasim NH. Differential expression of basal microRNAs’ patterns in human dental pulp stem cells. J Cell Mol Med. 2015;19(3):566-80.

287. Hu D, Young NM, Xu Q, Jimmichzy H, Green RM, Mio W, et al. Signals from the brain induce variation in avian facial shape. Dev Dyn. 2015; in press.

288. Schmidl C, Rendeiro AV, Sheffield NC, Bock C. ChIP-Seq analysis of chromatin remodeling at the 3'-end of histone-marked genes. Cell Stem Cell. 2016;165(7):1586-97.

289. Kloet SL, Makowski MM, Baymaz HI, van Voorthuijsen L, Karemaker ID, Santanach A, et al. Alcohol-induced suppression of KDM6B dysregulates the mineralization potential in dental pulp stem cells. Mech Ageing Dev. 2015;60(11):1613-7.
stem-cell differentiation. Nat Struct Mol Biol. 2016;23(7):682-690.

300. Hughes TE, Townsend GC, Pinkerton SK, Bockmann MR, Seow WK, Brook AH, et al. The teeth and faces of twins: providing insights into dentofacial development and oral health for practising oral health professionals. Aust Dent J. 2014;59 Suppl 1:101-16.