Epigenetic Control of Osteogenic Lineage Commitment

Martin Montecino1*, Margarita E. Carrasco1 and Gino Nardocci2,3

1 Faculty of Medicine and Faculty of Life Sciences, Institute of Biomedical Sciences and FONDAP Center for Genome Regulation, Universidad Andres Bello, Santiago, Chile, 2 Faculty of Medicine, Universidad de los Andes, Santiago, Chile, 3 Molecular Biology and Bioinformatic Lab, Program in Molecular Biology and Bioinformatics, Center for Biomedical Research and Innovation (CII), Universidad de los Andes, Santiago, Chile

INTRODUCTION

Osteoblast lineage commitment is regulated by a coordinated set of extra cellular stimuli and developmentally-regulated signaling pathways, including those mediated by bone morphogenic proteins (BMPs), Wnt-ligands, steroid hormones, and growth factors, among others (Li et al., 1998; Nishimura et al., 1998; Drissi et al., 2000; Yamaguchi et al., 2000; Zhang et al., 2008). Following activation in pre-osteogenic cells, these signaling pathways modulate the expression and function of osteoblast master transcription factors, which in turn control the expression of downstream bone-phenotypic genes, thus establishing the osteoblastic cell component of the mammalian skeleton (Stein et al., 2004; Long, 2012). Although the precise molecular mechanisms associated with transcriptional control of these osteoblast-related genes are still far from being completely understood, significant progress has been made during the last two decades.

We discuss research demonstrating the role of epigenetic mechanisms in controlling gene transcription during mesenchymal cell commitment to the osteogenic lineage. We first overview key components of epigenetic mechanisms that control gene expression in mammals and then...
describe how these epigenetic processes contribute to regulate transcription of the Runx2 and Sp7 genes, two critical osteogenic master regulators.

**GENERAL OVERVIEW OF EPIGENETIC CONTROL IN MAMMALS**

Nuclear DNA is organized as chromatin. Nucleosomes are the fundamental units of this chromatin and are composed of an approximately 147 bp DNA segment wrapped around an octamer of histone proteins (two each of histones H2A, H2B, H3, and H4) (Ramakrishnan, 1997; Richmond and Davey, 2003). A portion of these histone proteins (N-terminus tail) extends beyond the limits of the particle core, providing additional surfaces for interaction with nuclear proteins that regulate transcription (Strahl and Allis, 2000; Bannister and Kouzarides, 2011; Voigt et al., 2013). As specific residues (e.g., lysines and arginines) within these histone tails are subject to extensive enzymatic post-translational modifications, the potential for modifying the chemical environment surrounding the chromatin fibers represents a key regulatory component during gene expression control (Bannister and Kouzarides, 2011). The genomic DNA can be also enzymatically modified affecting the degree of compaction of chromatin. Increased condensation reduces the possibility that specific regulatory DNA sequence motifs are recognized by transcription factors that control transcription (Franchini et al., 2012; Bogdanović and Lister, 2017).

Molecular components that regulate chromatin organization and transcription are critical players during cell differentiation. Among them the large group of “histone post-translational modifications” (HPTMs), which are considered a principal “epigenetic” mechanism (Bannister and Kouzarides, 2011; Shilatifard, 2012; Voigt et al., 2013). HPTMs may function as docking sites on the chromatin fiber surface that can be recognized by regulatory proteins (“epigenetic readers”) that contain specific complementary domains (e.g., chromodomains interact with methylated histone lysine residues) (Voigt et al., 2013; Venkatesh and Workman, 2015). A number of protein complexes that are capable of mediating deposition (“epigenetic writers”) or elimination (“epigenetic erasers”) of HPTMs have been identified in eukaryotic cells. Importantly, most of the core subunits of these complexes are evolutionary conserved (Dimitrova et al., 2015; Piunti and Shilatifard, 2016; Jambhekar et al., 2019), indicating that their functions during gene expression control are also conserved across species.

Genetic studies allowed the identification of the Polycomb Group (PcG) and the Trithorax Group (TrxG) complexes, which mediate inhibition and activation of transcription, respectively, during embryogenesis (Ringrose and Paro, 2007). One signature property of the evolutionary conserved PcG complexes PRC1 and PRC2 (Polycomb Repressive Complex 1 and 2) is to mediate the formation of repressed chromatin. In mammals, PRC2 includes as main subunits Enhancer of Zeste Homolog 2 or 1 (Ezh2/Ezh1), Suppressor of Zeste 12 (Su(z)12), and Embryonic Ectoderm Development (Eed) (Piunti and Shilatifard, 2016; Yu et al., 2019). Ezh2 (or alternatively Ezh1) is the main catalytic component of PRC2 mediating tri-methylation of the lysine 27 residue of histone H3 (H3K27me3), modification that is associated with transcriptionally silent chromatin (Voigt et al., 2013; Piunti and Shilatifard, 2016).

Several mammalian TrxG complexes, including COMPASS (Complex of Proteins Associated with Set1a/b) and the Mixed Lineage Leukemia (MLL1–5)-containing COMPASS-like complexes have been reported (Shilatifard, 2012). TrxG-mediated activity involves mono-, di-, and tri-methylation of the lysine 4 residue of histone H3 (H3K4me1, H3K4me2, and H3K4me3). H3K4me3 is often found enriched at transcriptionally active chromatin (euchromatin), mainly around the transcription start sites (TSSs) of promoters. Moreover, H3K4me3 can be recognized by the RNA polymerase II complex, hence facilitating transcriptional activity at H3K4me3-marked gene promoters (Vermeulen et al., 2007). Set1-COMPASS and MLL-COMPASS-like complexes include the Wdr5 (WD Repeat Domain 5) subunit which has been shown necessary for assembly, stability and optimal enzymatic activity of these complexes (Shilatifard, 2012). Interestingly, some of these histone-methyllating complexes also include enzymatic activities that can remove other histone marks. For instance, MLL3/4-COMPASS-like that also contains the H3K27me3 demethylase Utx/Kdm6a (Piunti and Shilatifard, 2016). Hence, binding of MLL3/4-COMPASS-like to a particular genomic region can also result in reduced H3K27me3, an epigenetic signature associated with decreased transcription (Voigt et al., 2013; Piunti and Shilatifard, 2016).

Set1-COMPASS mediates global genomic deposition of H3K4me3 in most mammalian cells and therefore its function is tightly associated with transcriptional activation of a large number of genes. MLL2-COMPASS-like has been shown responsible for H3K4me3 deposition at promoters in embryonic stem cells (ESCs), contributing to transcriptional upregulation of homeobox genes (Denissov et al., 2014). Interestingly, MLL3/4-COMPASS-like can catalyze the deposition of H3K4me1 at enhancer elements in mammalian cells (Hu et al., 2013; Yan et al., 2018). Recent results from several teams, however, also show that MLL3/4 complexes can mediate the maintenance of the H3K4me1 mark at proximal promoter regions of repressed, but poised for expression, genes (Cheng et al., 2014; Rojas et al., 2015, 2019; Aguilar et al., 2016; Sepulveda et al., 2017a; Local et al., 2018). Moreover, MLL3/4 depletion results in transcriptional activation of some MLL3/4-target genes as both Set1a/b-COMPASS and MLL1-COMPASS-like gain access to these genes to mediate the transition from H3K4me1 to H3K4me3 that accompanies transcription (Cheng et al., 2014). Together, these studies imply that different COMPASS and COMPASS-like complexes bind to target sequences in a highly coordinately manner to first maintain a gene silent, but poised for transcription, and subsequently to activate its expression.

Chromatin domains with decreased enrichment of H3K4me3 or H3K27me3 can also be maintained through the activity of lysine demethylases (Dimitrova et al., 2015; Jambhekar et al., 2019). In particular, demethylation of H3K4me3 in mammals is mediated by members of the Jarid1/Kdm5 family.
(Jarid1a, b, c, and d) (Kooistra and Helin, 2012), which convert H3K4me3 and H3K4me2 to H3K4me1 (Christensen et al., 2007; Iwase et al., 2007; Yamane et al., 2007). Absence of these enzymes can result in enrichment of H3K4me3 at target genes and transcriptional activation (Albert et al., 2013; Rojas et al., 2015). On the other hand, histone demethylases Utx/Kdm6a and Jmjd3/Kdm6b can catalyze the removal of methyl groups from H3K27me3 and therefore counteract the silencing activity of PRC2 (Agger et al., 2007; Hong et al., 2007; Lan et al., 2007; Santa et al., 2007; Xiang et al., 2007).

Methylation at H3K9 (H3K9me1, H3K9me2, and H3K9me3) is strongly associated with the formation of highly compacted and transcriptionally repressed chromatin (heterochromatin) (Saksouk et al., 2015; Nicetto and Zaret, 2019). The methyl-transferases that deposit this modification (“H3K9me writers”) include Suv39H1/Kmt1a, Suv39H2/Kmt1b and Setdb1/ESET/Kmt1e, which can mediate mono-, di-, and tri-methylation. Also, G9a/Ehmt2/Kmt1c and GLP/Ehmt1/Kmt1d, which mediate H3K9me1 and H3K9me2 (Saksouk et al., 2015; Nicetto et al., 2019). All of these enzymes are critical components of several transcription repressive complexes that operate during cell differentiation (Nicetto et al., 2019). The H3K9 methyl-transferase activity is counteracted by H3K9 demethylases, that remove these marks (“H3K9me erasers”) (Jansen et al., 2018). Among them, Lsd1/Kdm1, Jmjd1a/Kdm3a, Jmjd1c/Kdm3c, which can eliminate H3K9me1 or H3K9me2, and Jmjd2a/Kdm4a, Jmjd2b/Kdm4b, Jmjd2c/Kdm4c, and Jmjd2d/Kdm4d, which erase H3K9me1, H3K9me2, and H3K9me3.

Chromatin organization and transcriptional activity is also mediated by ATP-dependent remodelers (Horn and Peterson, 2001; Bakshi et al., 2010; Längst and Manelyte, 2015), which are multi-subunit complexes that include a catalytic core (e.g., Brg1 in the mammalian SWI/SNF and INO80 in the INO80 complex) mediating binding and hydrolysis of ATP (ATPase activity) (Tsukiyama, 2002; Martens and Winston, 2001; Bakshi et al., 2010; Längst and Manelyte, 2015), which modulate histone arginine methylation (Kanno et al., 2004; Pal et al., 2004). This regulation is due to the presence of bromo- and chromo-domains at subunits of these complexes (e.g., SWI/SNF and CHD3/4) that recognize these modified histone residues (Becker and Workman, 2013).

Genomic DNA can be methylated at cytosines that are followed by guanosines (CpG dinucleotides). The DNA methyl-transferases that mediate this modification belong to a well-conserved family of proteins that include both maintenance (Dnmt1) and de novo (Dnmt3a and Dnmt3b) activities (Franchini et al., 2012; Bogdanović and Lister, 2017). Most reports demonstrate that methylated CpG is associated with higher chromatin compaction and reduced transcriptional activity (Franchini et al., 2012; Bogdanović and Lister, 2017). DNA demethylation in mammalian cells involves the conversion from 5-methyl-CpG (5mCpG) to 5-hydroxymethyl-CpG (5hmCpG) by the activity of the Ten Eleven Transformation (Tet) family of dioxygenases (Tahiliani et al., 2009; Ito et al., 2010; Bogdanović and Lister, 2017). Importantly, Tet proteins form regulatory complexes with chromatin remodelers like SWI/SNF as well as with histone methyl-transferases and histone demethylases (Williams et al., 2011; Yildirim et al., 2011; Neri et al., 2013).

Enrichment of H3K9me3/2 at transcriptionally-inactive chromatin can be associated with the presence of 5mCpG. This is due to the ability of the proteins that “write” and “read” these two epigenetic marks to form complexes (Janssen et al., 2018; Nicetto and Zaret, 2019) thereby providing a means for both repressive epigenetic modifications (5mCpG and H3K9me3) to collaborate in generating a condensed chromatin structure that reduces transcription. These findings further indicate that different epigenetic mechanisms leading to chromatin remodeling and transcriptional control can function in a coordinated and complementary manner within eukaryotic cells, allowing an effective regulation of gene expression in response to physiological cues.

EPIGENETIC CONTROL OF THE EXPRESSION OF MASTER REGULATORS OF OSTEOGENESIS

Differentiation of mesenchymal stem cells (MSCs) to the osteogenic lineage requires the expression and function of two master transcription factors: Runx2 (Runt Related Transcription Factor 2) and Sp7 (also known as Osterix). These factors bind to and control the expression of numerous downstream target genes that code for proteins that are necessary for establishing the osteoblast phenotype (Zhang, 2010; Sinha and Zhou, 2013; Meyer et al., 2014; Wu et al., 2014). Runx2 and Sp7 are expressed at early embryonic stages of the mesenchyme-osteoblast lineage commitment process and optimal concentrations of both factors in pre-osteogenic cells is essential for proper osteoblast differentiation (Long, 2012). Runx2 binds to and activates the Sp7 promoter (Yoshida et al., 2012), indicating that Runx2 is an upstream transcription factor during osteogenesis, but that this differentiation process requires the expression of both master regulators (Long, 2012). In recent years, several groups have demonstrated that changes in chromatin structure and expression of the Runx2 and Sp7 genes are epigenetically-controlled (Yang et al., 2013, 2015; Tai et al., 2014; Dudakovíc et al., 2015; Rojas et al., 2015, 2019; Zhang et al., 2015; Aguilar et al., 2016, 2020; Park-Min, 2016; Zhou et al., 2016; Sepulveda et al., 2017a; Cakouros and Grontos, 2020; Figure 1).
Methylation at Histone H3K4 and H3K27 Residues

Active transcription of the Runx2 and Sp7 genes is accompanied by an epigenetic landscape that includes increased H3K4me3 enrichment at both promoter regions (Figure 1). Hence, transcription of these master genes requires binding and activity of COMPASS and COMPASS-like complexes (Shilatifard, 2012). Studies initiated two decades ago first demonstrated
that over-expression of Wdr5, a critical subunit of these complexes, accelerates osteoblast and chondrocyte differentiation programs (Gori et al., 2001, 2006; Zhu et al., 2008). Wdr5 binds to the Runx2-P1 promoter in osteoblastic cells (Rojas et al., 2015) where it can mediate transcriptional upregulation. It was recently found that transcription of both Runx2 and Sp7 during osteogenesis requires that Set1-COMPASS and MLL2-COMPASS-like complexes mediate deposition of the H3K4me3 mark at both promoters (Rojas et al., 2015, 2019; Sepulveda et al., 2017b).

It has been determined that an enrichment of H3K4me1 accompanies transcriptional silence at the Runx2 and Sp7 genes in non-osteoblastic and mesenchymal uncommitted cells (Rojas et al., 2015; Aguilar et al., 2016, 2020; Sepulveda et al., 2017a,b). As MLL3/4-COMPASS can mediate maintenance of H3K4me1 at repressed, but poised for expression, genes (Cheng et al., 2014; Rojas et al., 2015, 2019; Aguilar et al., 2016; Sepulveda et al., 2017a; Local et al., 2018), one possibility is that H3K4me1 is deposited at the Runx2 and Sp7 promoters by the MLL3/4 complexes that have been found associated with both genes in MSCs prior to engage osteogenesis (Sepulveda et al., 2017b; Rojas et al., 2019).

Maintenance of chromatin with low H3K4me3 enrichment (and hence elevated H3K4me1) at the Runx2 promoter can be also achieved by binding and activity of the H3K4 demethylase Jarid1b/Kdm5b (Figure 1; Rojas et al., 2015, 2019; Bustos et al., 2017; Sepulveda et al., 2017a), which converts H3K4me3 to H3K4me1 (Christensen et al., 2007; Iwase et al., 2007; Yamane et al., 2007). Targeted depletion of Jarid1b/Kdm5b in mice results in elevated lethality at early post-natal stages, exhibiting these animals cranial dysmorphic parameters and skeletal alterations (Albert et al., 2013). Moreover, silencing of Runx2 expression during myogenic cell differentiation is mediated by Jarid1b/Kdm5b bound to the Runx2 promoter (Rojas et al., 2015, 2019). Accordingly, knockdown of Jarid1b/Kdm5b expression is accompanied by enrichment of H3K4me3 at the Runx2 promoter and active Runx2 transcription in mouse mesenchymal pluripotent cells differentiating to non-osteoblastic lineages (Rojas et al., 2015; Bustos et al., 2017; Sepulveda et al., 2017a).

Findings from several teams revealed that regulating the repressive mark H3K27me3 represents an important step during the differentiation of MSCs toward osteoblasts. It was demonstrated that the activity of the H3K27 demethylases Utx/Kdm6a and Jmjd3/Kdm6b is critical for erasing this mark from the promoter of the Runx2 and Sp7 genes (Figure 1) and hence to induce their expression during osteogenesis (Ye et al., 2012; Hemming et al., 2014; Rojas et al., 2015; Park-Min, 2016; Sepulveda et al., 2017b; Cakouros and Gronthos, 2020; Sen et al., 2020). Additionally, an increased activity of the PRC2 complex that “writes” the H3K27me3 mark, can significantly limit the ability of MSCs to engage osteogenic differentiation (Wei et al., 2010). Moreover, forced expression of Eh2 in pre-osteoblastic cells results in significant downregulation of osteogenic genes, including Runx2 and its downstream targets (Hemming et al., 2014). Accordingly, knockdown of Eh2 expression in MSCs and pre-osteoblastic cells results in increased expression of the Runx2 (Rojas et al., 2015, 2019; Hemming et al., 2016) and Sp7 (Sepulveda et al., 2017b) genes.

**Methylation at the Histone H3K9 Residue**

The repressive mark H3K9me3 plays an important role during differentiation of MSCs to the osteoblastic lineage (Ye et al., 2012, 2018; Lawson et al., 2013; Tai et al., 2014; Rojas et al., 2015; Zhang et al., 2015; Sepulveda et al., 2017a,b). H3K9me3 is found enriched at the Runx2 and Sp7 promoters in cells that do not express these genes (Figure 1) and binding of proteins that can write and read this mark has been detected in non-osseous cells (Zhang et al., 2015; Aguilar et al., 2016; Sepulveda et al., 2017b). The H3K9 methyl transferase Setdb1/ESET/Kmt1c binds to the Sp7 promoter and maintains a H3K9me3-enriched transcriptionally-silent environment in mouse MSCs and other non-osseous cells (Sepulveda et al., 2017b). Accordingly, during BMP2-induced osteogenic differentiation of MSCs, Setdb1/ESET/Kmt1c is released from this promoter and replaced by the H3K9me3 demethylase Jmjd2a/Kdm4a, an exchange that is accompanied by decreased enrichment of H3K9me3 and transcription of the Sp7 gene (Sepulveda et al., 2017b). Interestingly, it was also established that a different member of this family of demethylases, Jmjd2b/Kdm2b, plays a critical role during formation of the mouse skeleton (Ye et al., 2012, 2018). However, this demethylase neither directly binds nor decreases the H3K9me3 enrichment at the Sp7 and Runx2 promoters (Ye et al., 2012, 2018).

Although most studies support the role of the H3K27me3 and H3K9me3 marks during repression of both master osteogenic genes, recent analyses indicate that for certain cells, this role may have to be re-evaluated. Thus, analysis of uncommitted human umbilical cord-derived Wharton Jelly MSCs (WJ-MSCs) showed that the Runx2-P1 promoter region does not exhibit significant enrichment of H3K9me3 and H3K27me3 (Bustos et al., 2017; Sepulveda et al., 2017a). Interestingly, both marks are detected at the Sp7 gene promoter in these cells and represent a relevant component of the epigenetic barrier preventing the expression of Sp7 when the cells are induced to differentiate to the osteoblastic lineage (Bustos et al., 2017; Sepulveda et al., 2017a). Hence additional mechanisms are preventing the expression of Runx2-p57 in these uncommitted WJ-MSCs in the absence of H3K27me3 and H3K9me3. One proposed component is the H3K4 demethylase Jarid1b/Kdm5b which was found enriched at this Runx2 P1 promoter concomitant with elevated H3K4me1 (Bustos et al., 2017; Sepulveda et al., 2017a). Knockdown of Jarid1b/Kdm5b expression or selective inhibition of Jarid1b/Kdm5b activity using small molecules, is accompanied by enrichment of H3K4me3 at the Runx2 promoter and Runx2 upregulation in these WJ-MSCs (Bustos et al., 2017; Sepulveda et al., 2017a). Future studies need to carefully address what alternative mechanisms control the expression of Runx2 and Sp7 in the different sources of human MSCs available.

**Histone H3 and H4 Acetylation**

Histone lysine acetylation also plays a relevant role during bone formation as it supports increased expression of critical genes for osteoblast differentiation (Schroeder and Westendorf, 2005;
remodeling and transcription of the Runx2 gene is accompanied by acetylation of histones H3 and H4 (Figure 1) in nucleosomes associated with the P1 promoter region (Lee H. W. et al., 2006; Hassan et al., 2007; Cruzat et al., 2009; Gordon et al., 2011). This acetylation is correlated with decreased expression of HDAcs (HDAC1, 2, and 3) during BMP2-induced osteoblast differentiation (McGee-Lawrence and Westendorf, 2011). Accordingly, knockdown of HDAC1 and HDAC3 expression facilitates osteogenic differentiation (Maroni et al., 2012) and a number of HDAC inhibitors [Sodium Butyrate, Trichostatin A, Valproic acid, and Suberoyl Anilide Hydroxamic Acid (SAHA)] can promote osteogenesis in models including primary calvarial cells and MSCs (Cho et al., 2005; Schroeder and Westendorf, 2005; Lee H. W. et al., 2006; Haberland et al., 2010; Hu et al., 2012; Dudakov et al., 2013; Zych et al., 2013). Moreover, the histone acetyl transferase inhibitor 5-Azacytidine (5-Aza) promotes osteogenesis in models including primary calvarial cells and MSCs (BM-MSCs), Tet1 is pre-bound to osteogenic related gene expression. Future studies will need to consider the influence of the mechanical environments at which MSCs are maintained and differentiated ex vivo. Recent results demonstrate that the substrate stiffness at which hMSCs are grown in culture can induce rapid and stable changes in chromatin organization and the associated epigenetic landscapes that permanently up- or down-regulate genes (Killaars et al., 2019), hence modifying the ability of these cells to engage osteogenesis. Future research also needs to develop approaches that include safe delivery strategies that limit undesired effects of treatments based on epigrugs on non-target tissues and organs, thereby decreasing potential negative secondary effects in treated patients.
Gori, F., Friedman, L. G., and Demay, M. B. (2006). WDR5, a WD-40 protein, regulates osteoblast differentiation during embryonic bone development. Dev. Biol. 295, 498–506. doi: 10.1016/j.ydbio.2006.02.031

Haberland, M., Carrer, M., Mokalled, M. H., Montgomery, R. L., and Olson, E. N. (2010). Redundant control of adipogenesis by histone deacetylases 1 and 2. J. Biol. Chem. 285, 14663–14670. doi: 10.1074/jbc.m109.081679

Hassan, M. Q., Tare, R., Lee, S. H., Mandeville, M., Weiner, B., Montecino, M., et al. (2007). HOXA10 controls osteoblastogenesis by directly activating bone regulatory and phenotypic genes. Mol. Cell. Biol. 27, 3337–3352. doi: 10.1128/mcb.01244-06

Hemmings, S., Cakouros, D., Jemseman, S., Cooper, L., Menicanin, D., Zannettino, A., et al. (2014). EZH2 and KDM6A Act as an epigenetic switch to regulate mesenchymal stem cell lineage specification. Stem Cells 32, 802–815. doi: 10.1002/stem.1573

Hemmings, S. E., Cakouros, D., and Vandyke, K. (2016). Identification of novel EZH2 targets regulating osteogenic differentiation in mesenchymal stem cells. Stem Cells Dev. 25, 909–921. doi: 10.1089/scd.2015.0384

Hong, S., Cho, Y.-W., Yu, I.-R., Yu, H., Veenstra, T. D., and Ge, K. (2007). Identification of JmJ-C domain-containing UTX and JMJ3 as histone H3 lysine 27 demethylases. Proc. Natl. Acad. Sci. U.S.A. 104, 18439–18444. doi: 10.1073/pnas.0707292104

Horn, P. J., and Peterson, C. L. (2001). The bromodomain: a regulator of ATP-dependent chromatin remodeling! Front. Biosci. 6, D1019–D1023. doi: 10.2741/horn

Hu, D., Gao, X., Morgan, M. A., Herz, H.-M., Smith, E. R., and Shilatifard, A. (2013). The MLL3/MLL4 branches of the COMPASS family function as major histone H3K4 monomethyltransferases. Mol. Cell. Biol. 33, 4745–4754. doi: 10.1128/mcb.01181-13

Hu, X., Zhang, X., Dai, L., Zhu, J., Jia, Z., Wang, W., et al. (2012). Histone deacetylase inhibitor trichostatin A promotes the osteogenic differentiation of rat adipose-derived stem cells by altering the epigenetic modifications on Runx2 promoter in a BMP signaling-dependent manner. Stem Cells Dev. 21, 248–255. doi: 10.1089/scd.2012.0105

Ito, S., D’Alessio, A. C., Tanarana, O. V., Hong, K., Sowers, L. C., and Zhang, Y. (2010). Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 466, 1129–1133. doi: 10.1038/nature09303

Iwase, S., Lan, F., Bayliss, P., de la Torre-Ubieta, L., Huarte, M., Qi, H. H., et al. (2007). The X-linked mental retardation gene SMCRX/JARID1C defines a family of histone H3 lysine 4 demethylases. Cell 128, 1077–1088. doi: 10.1016/j.cell.2007.02.017

Jambhekar, A., Dhall, A., and Shi, Y. (2019). Roles and regulation of histone methylation in animal development. Nat. Rev. Mol. Cell. Biol. 20, 625–641. doi: 10.1038/s41580-019-0151-1

Janssen, A., Colmenares, S. U., and Karpen, G. H. (2018). Heterochromatin: guardian of the genome. Annu. Rev. Cell Dev. Biol. 34, 265–288. doi: 10.1146/annurev-cellbio-100616-062653

Kanno, T., Kanno, Y., Siegel, R. M., Jang, M. K., Lenardo, M. J., and Ozato, K. (2004). Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. Mol. Cell. 13, 33–43. doi: 10.1016/s1097-2765(03)00482-9

Killaars, A., Grim, J. C., Walker, C. J., Hushka, E. A., Brown, T. E., and Anseth, K. S. (2019). Extended exposure to stiff microenvironments leads to persistent downregulation of the guardian of the genome. Mol. Cell. Biol. 39, 689–694. doi: 10.1128/mcb.00877-19

Kilarski, A., Gruninger, M., and Helm, K. (2014). The JmjC domain-containing histone H3 lysine 27 demethylases. Mol. Cell. 54, 225–236. doi: 10.1016/j.molcel.2014.02.032

Kilarski, A., Gruninger, M., and Helm, K. (2014). The JmjC domain-containing histone H3 lysine 27 demethylases. Mol. Cell. 54, 225–236. doi: 10.1016/j.molcel.2014.02.032

Kilarski, A., Gruninger, M., and Helm, K. (2014). The JmjC domain-containing histone H3 lysine 27 demethylases. Mol. Cell. 54, 225–236. doi: 10.1016/j.molcel.2014.02.032
Rojas, A., Sepulveda, H., Henriquez, B., Aguilar, R., Opazo, T., Nardocci, G., Richmond, T. J., and Davey, C. A. (2003). The structure of DNA in the nucleosome. Nature 423, 187–189. doi: 10.1038/nature01959

Ringrose, L., and Paro, R. (2007). Polycistronic/Trithorax response elements and epigenetic memory of cell identity. Development 134, 223–232. doi: 10.1242/dev.02723

Rojas, A., Aguilar, R., Henriquez, B., Lian, J. R., Stein, I. J., Stein, G. S., et al. (2015). Epigenetic control of the bone-masterrunx2 gene during osteoblast-lineage commitment by the histone demethylase JARID1B/KDM5B. J. Biol. Chem. 290, 28323–28342. doi: 10.1074/jbc.M115.657825

Rojas, A., Sepulveda, H., Henriquez, B., Aguilar, R., Ozapo, T., Nardocci, G., et al. (2019). Mi-COMPASS complexes mediate H3K4me3 enrichment and transcription of the osteoblast master gene Runx2/p57 in osteoblasts. J. Cell. Physiol. 243, 6244–6253. doi: 10.1002/jcp.27355

Ruijtenberg, S., and van den Heuvel, S. (2016). Coordinating cell proliferation and differentiation: Antagonism between cell cycle regulators and cell type-specific gene expression. Cell Cycle 15, 196–212. doi: 10.1080/15384101.2015.1120925

Salskoum, N., Simboeck, E., and Dejaer, J. (2015). Constitutive heterochromatin formation and transcription in mammals. Epigenet. Chromatin. 8:3. doi: 10.1186/s13057-015-0019-8

Santa, F. D., Totaro, M. G., Prosperini, E., Notarbartolo, S., Testa, G., and Naloti, G. (2007). The histone H3 lysine-27 demethylase jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. Cell 130, 1083–1094. doi: 10.1016/j.cell.2007.08.019

Schroeder, T. M., and Westendorf, J. J. (2005). Histone deacetylase inhibitors promote osteoblast maturation. J. Bone. Miner. Res. 20, 2254–2265. doi: 10.1359/jbmr.050813

Sen, B., Paradise, C. R., Xie, Z., Sankaran, J., Uzer, G., Stynner, M., et al. (2020). β-catenin preserves the stem state of murine bone marrow stromal cells through activation of EZH2. J. Bone. Miner. Res. 35, 1149–1162. doi: 10.1002/jbmr.3975

Sepulveda, H., Aguilar, R., Prieto, C. P., Bustos, F. A., Fau, S., Lattus, J., et al. (2017a). Epigenetic signatures at the RUNX2-P1 and Sp7 gene promoters control osteogenic lineage commitment of umbilical cord-derived mesenchymal stem cells. J. Cell. Physiol. 232, 2519–2527. doi: 10.1002/jcp.25627

Sepulveda, H., Villagra, A., and Montecino, M. (2017b). Tet-mediated DNA demethylation is required for SWI/SNF-dependent chromatin remodeling and histone-modifying activities that trigger expression of the Sp7 osteoblast master gene during mesenchymal lineage commitment. Mol. Cell. Biol. 37:e00177-17. doi: 10.1128/mcb.00177-17

Serna, I. L., de la Carlson, K. A., and Imbalzano, A. N. (2001). Mammalian SWI/SNF complexes promote MyoD-mediated muscle differentiation. Nat. Genet. 27, 187–190. doi: 10.1038/348426

Shilatifard, A. (2012). The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. Annu. Rev. Biochem. 81, 65–95. doi: 10.1146/annurev-biochem-051710-134100

Sinha, K. M., and Zhou, X. (2013). Genetic and molecular control of ostein in skeletal formation. J. Cell. Biol. 114, 975–984. doi: 10.1083/jcb.24439

Srivastava, S., Mishra, R. K., and Dhawan, J. (2010). Regulation of cellular chromatin state: insights from quiescence and differentiation. Organogenesis 6, 37–47. doi: 10.4161/organ.6.1.11337

Stein, G. S., Lian, J. B., van Wijnen, A. J., Stein, J. L., Montecino, M., Javed, A., et al. (2004). Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene 23, 4315–4329. doi: 10.1038/sj.onc.1207676

Strahl, B. D., and Allis, C. D. (2000). The language of covalent histone modifications. Nature 403, 41–45. doi: 10.1038/35074112

Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., et al. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 323, 930–935. doi: 10.1126/science.1170116

Tai, P. W., Wu, H., Gordon, J. A. R., Whitfield, T. W., Barutcu, A. R., van Wijnen, A. J., et al. (2014). Epigenetic landscape during osteoblastogenesis defines a differentiation-dependent Runx2 promoter region. Gene 550, 1–9. doi: 10.1016/j.ygeno.2014.05.004

Tsukiyama, T. (2002). The in vivo functions of AT-dependent chromatin-remodelling factors. Nat. Rev. Mol. Cell Biol. 3, 422–429. doi: 10.1038/nrm828

Venkatesh, S., and Workman, J. L. (2015). Histone exchange, chromatin structure and the regulation of transcription. Nat. Rev. Mol. Cell. Biol. 16, 178–189. doi: 10.1038/nr63941

Vermeulen, M., Mulder, K. W., Denissen, S., Pijnappel, W. W. M. P., van Schaik, F. M. A., Varier, R. A., et al. (2007). Selective anchoring of TFIID to nucleosomes by inhibition of histone H3 lysine 4. Cell 131, 58–69. doi: 10.1016/j.cell.2007.08.016

Voigt, P., Teer, W.-W., and Reinberg, D. (2013). The double take on bivalent promoters. Genes Dev. 27, 1318–1338. doi: 10.1101/gad.219626.113

Wei, Y., Chen, Y.-H., Li, L.-Y., Lang, J., Yeh, S.-P., Shi, B., et al. (2010). CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. Nat. Cell Biol. 13, 87–94. doi: 10.1038/ncb2139
Montecino et al. Epigenetics of Osteoblast Differentiation

Wu, H., Whitfield, T. W., Gordon, J. A. R., Dobson, J. R., Tai, P. W. L., van Ye, L., Fan, Z., Yu, B., Chang, J., Hezaimi, K. A., Zhou, X., et al. (2018). Histone demethylase

Yamaguchi, A., Komori, T., and Suda, T. (2000). Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Chaf1. Endocr. Rev. 21, 393–411. doi: 10.1210/edrv.21.4.0403

Yamane, K., Tateishi, K., Klose, R. J., Fang, J., Fabrizio, L. A., Erdjument-Bromage, H., et al. (2007). PLU-1 Is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. Mol. Cell. 25, 801–812. doi: 10.1016/j.molcel.2007.03.001

Yan, J., Chen, S.-A. A., Local, A., Liu, T., Qiu, Y., Dorighi, K. M., et al. (2018). Histone H3 lysine 4 monomethylation modulates long-range chromatin interactions at enhancers. Cell Res. 28, 204–220. doi: 10.1038/cr.2018.1

Yang, D., Okamura, H., Nakashima, Y., and Haneji, T. (2013). Histone demethylase JmjD3 regulates osteoblast differentiation via transcription factors Runx2 and osterix. J. Biol. Chem. 288, 33530–33541. doi: 10.1074/jbc.m113.497040

Yang, D., Okamura, H., Teramachi, J., and Haneji, T. (2015). Histone demethylase Utx regulates differentiation and mineralization in osteoblasts. J. Cell. Biochem. 116, 2628–2636. doi: 10.1002/jcb.25210

Ye, L., Fan, Z., Yu, B., Chang, J., Hezaimi, K. A., Zhou, X., et al. (2012). Histone demethylase KDM4B and KDM6B promotes osteogenic differentiation of human MSCs. Cell Stem Cell 11, 50–61. doi: 10.1016/j.stem.2012.04.009

Ye, L., Fan, Z., Yu, B., Chang, J., Hezaimi, K. A., Zhou, X., et al. (2018). Histone demethylases KDM4B and KDM6B promote osteogenic differentiation of human MSCs. Cell Stem Cell 23, 898–899. doi: 10.1016/j.stem.2018.11.002

Yildirim, O., Li, R., Hung, J.-H., Chen, P. B., Dong, X., Ee, L.-S., et al. (2011). Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells. Cell 147, 1498–1510. doi: 10.1016/j.cell.2011.11.054

Yoshida, C. A., Komori, H., Maruyama, Z., Miyazaki, T., Kawasaki, K., Furuiuchi, T., et al. (2012). SP7 inhibits osteoblast differentiation at a late stage in mice. PLoS One 7:e32364. doi: 10.1371/journal.pone.0032364

Yu, J.-R., Lee, C.-H., Oksuz, O., Stafford, J. M., and Reinberg, D. (2019). PRC2 is high maintenance. Genes Dev. 33, 903–935. doi: 10.1101/gad.325050.119

Zhang, C. (2010). Transcriptional regulation of bone formation by the osteoblast-specific transcription factor Osx. J. Orthop. Surg. Res. 5:37. doi: 10.1186/1749-799x-5:37

Zhang, Y., Hassan, M. Q., Xie, R. L., Hawse, J. R., Spelsberg, T. C., Montecino, M., et al. (2008). Co-stimulation of the bone-related Runx2 P1 promoter in mesenchymal cells by SP1 and ETS transcription factors at polymorphic purine-rich DNA sequences (Y-repeats). J. Biol. Chem. 284, 3125–3135. doi: 10.1074/jbc.m807466200

Zhang, Y.-X., Sun, H.-L., Liang, H., Li, K., Fan, Q.-M., and Zhao, Q.-H. (2015). Dynamic and distinct histone modifications of osteogenic genes during osteogenic differentiation. J. Biochem. 158, 445–457. doi: 10.1093/jb/miv059

Zhou, G.-S., Zou, J., Zou, S., and Li, X. (2016). INO80 is required for osteogenic differentiation of human mesenchymal stem cells. Sci. Rep. 6:35924. doi: 10.1038/srep35924

Zhou, G.-S., Zhang, X.-L., Wu, J.-P., Zhang, R.-P., Xiang, L.-X., Dai, L.-C., et al. (2009). 5-Azacytidine facilitates osteogenic gene expression and differentiation of mesenchymal stem cells by alteration in DNA methylation. Cytotechnology 60, 11–22. doi: 10.1007/s10616-009-9203-2

Zhu, E. D., Demay, M. B., and Gori, F. (2008). Wdr5 is essential for osteoblast differentiation. J. Biol. Chem. 283, 7361–7367. doi: 10.1074/jbc.M107498200

Zych, J., Stimamiglio, M. A., Senegaglia, A. C., Brozman, P. S., Dallagiovanna, B., Goldenberg, S., et al. (2013). The epigenetic modifiers 5-aza-2′-deoxycytidine and trichostatin A influence adipocyte differentiation in human mesenchymal stem cells. Braz. J. Med. Biol. Res. 46, 405–416. doi: 10.1590/1414-431x20132893

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Montecino, Carrasco and Nardocci. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.