Published in final edited form as:

Bone. 2015 December ; 81: 739–745. doi:10.1016/j.bone.2015.03.011.

Chromatin modifiers and histone modifications in bone formation, regeneration, and therapeutic intervention for bone-related disease

Jonathan. A.R. Gordon^a, Janet S. Stein^a, Jennifer J. Westendorf^b, and Andre J. van Wijnen^b

^a Department of Biochemistry, University of Vermont, Burlington, Vermont, United States

^b Department of Orthopedic Surgery, Mayo Clinic, Rochester, Minnesota, United States

Abstract

Post-translational modifications of chromatin such as DNA methylation and different types of histone acetylation, methylation and phosphorylation are well-appreciated epigenetic mechanisms that confer information to progeny cells during lineage commitment. These distinct epigenetic modifications have defined roles in bone, development, tissue regeneration, cell commitment and differentiation, as well as disease etiologies. In this review, we discuss the role of these chromatin modifications and the enzymes regulating these marks (methyltransferases, demethylases, acetyltransferases, and deacetylases) in progenitor cells, osteoblasts and bone-related cells. In addition, the clinical relevance of deregulated histone modifications and enzymes as well as current and potential therapeutic interventions targeting chromatin modifiers are addressed.

Keywords

Epigenetics; Chromatin; Chromatin-modifiers; bone lineage cells; acetylation; methylation

Introduction

The study of epigenetics has been widely defined as any heritable changes in an organism that occur without direct alteration in DNA sequence. This broad definition encompasses a wide range of chemical modifications of proteins and DNA that can alter the structure and accessibility of chromatin in the nucleus leading to altered gene expression. A great deal of research has defined multiple epigenetic mechanisms regulating transcription factor and

Please address correspondence to: Dr. Jonathan Gordon, Department of Biochemistry, University of Vermont, Given Building, Room E210C, 89 Beaumont Drive, Burlington, VT 05405; Phone (802) 656-4885.

Jonathan A R Gordon, University of Vermont, Department of Biochemistry, Burlington Vermont, United States;

Jonathan.A.Gordon@uvm.edu

Janet S Stein, University of Vermont, Department of Biochemistry, Burlington Vermont, United States; Janet.Stein@uvm.edu

Jennifer J Westendorf : Mayo Clinic, Orthopedic Surgery, Rochester, Minnesota, United States; Westendorf.Jennifer@mayo.edu

Andre J van Wijnen Mayo Clinic, Orthopedic Surgery, Rochester, Minnesota, United States; vanwijnen.andre@mayo.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures: The authors declare no conflict of interest

transcriptional machinery access to chromatin, including nucleosome positioning [1, 2], chromatin-chromatin interactions, lncRNA and miRNA binding [3, 4], and several unique chemical post-translational modifications to DNA and DNA-associated proteins [5]. As these epigenetic mechanisms are too complicated to cover in brief, this review will focus on the two most prominent (and therefore widely studied) epigenetic modifications; DNA methylation and post-translational modifications of histone proteins (i.e. lysine acetylation and methylation, and serine phosphorylation) as they relate to bone development, regeneration and disease.

DNA methylation

Methylation of cytosine bases at the fifth carbon (5-methylcytosine or 5mC) is a highly conserved DNA modification found in all mammals [6]. In somatic cells, cytosine methylation is essentially limited to palindromic CpG dinucleotides, however non-CpG methylation has been observed in some cells including; oocytes, pluripotent embryonic stem cells (ESCs), and mature neurons [7, 8]. DNA methylation is dynamically regulated during development and contributes to developmental stage- and cell-type specific epigenetic signals that profoundly impact gene expression [9]. Nearly 70% of annotated gene promoter regions and a large number of transcriptional start sites (TSS) are associated with short CpG-dense sequences [10]. This would suggest that a large number of genes are susceptible to methylation at these CpG islands (CGIs), however CGIs are frequently non-methylated, regardless of the transcriptional state of the corresponding genes [10]. There are several examples of CGI methylation during normal development resulting in stable silencing of gene transcription, however it would appear that CGI methylation may not be an initiating event in gene silencing, but rather acting to permanently maintain gene inactivation [10, 11]. Therefore at most genomic sites methylation patterns are static amongst specific tissues and change in cell-specific context as specialized cellular processes are activated or inactivated. Mechanistically, CpG methylation is thought to mediate gene silencing through direct inhibition of transcription factor binding due to DNA methylation or by recruiting methyl-binding domain proteins that in turn, recruit chromatin-modifying enzymes and complexes to methylated DNA.

Biological regulation of DNA methylation

Most CpG methylations are deposited in a coordinated effort by three conserved enzymes, DNA methyltransferase 1 (DNMT1), DNMT3A, and DNMT3B. During mitosis, DNMT1/UHRF1 recognizes methylated CpGs on the template strand and correspondingly deposits a methyl group on the nascent DNA [12]. Although maintenance of DNA methylation at mitosis ensures epigenetic inheritance at most genomic regions, there are many instances in which methylation must be actively targeted and others in which methylation must be inhibited or indirectly removed by exclusion of DNA methyltransferases. For instance, many CpG island promoters are unmethylated, but a subset of promoters is methylated during development (i.e. *de novo* methylation) or lineage commitment leading to gene inactivation. In these cases, DNMT3A and DNMT3B target promoters in complex with other epigenetic repressors, including histone deacetylases (HDACs) and methyltransferases, to remove H3K9 and/or H3K27 methylation and co-operatively repress gene activity [12]. The methylation status of CpG islands also allows for binding of regulatory complexes through

specific protein domains. The SRA domain in UHRF1 is able to recognize hemimethylated (mCG:GC) and methyl-CpG-binding domains (MBD) in proteins such as MECP2, MBD1, MBD2, and MBD4 are able to recognize symmetrically methylated (mCG:GCm) CpG dyads [13]. Several zinc finger-CxxC domain proteins including KDM2a (Fbxl11), KDM2b (Fbxl10), and MLL1 specifically recognize non-methylated DNA (CG:GC) and recruit chromatin-modifying activities to CpG islands [13]. Active DNA demethylation of CpG residues is initiated by the TET family of DNA dioxygenases. TET enzymes convert 5-methylcytosine to 5-hydroxymethylcytosine (5hmc) and subsequently 5-formylcytosine (5fc) and 5-carboxycytosine (5caC) leading to nucleotide or base excision repair [9].

DNA methylation in bone

The involvement of DNA methylation in musculoskeletal diseases has been most studied in the context of osteoarthritis. Studies have linked increased methylation at the MMP13 and iNOS promoters in human articular chondrocytes with the progression of arthritic disease [14-16]. Several studies have examined DNA methylation at specific gene promoters as a functional consequence or potential driver of osteoblast differentiation. Differentiation of human mesenchymal stem/stromal cells (MSCs) into the osteoblast lineage resulted in the progressive methylation of promoters associated with pluripotency/ stem cell genes [17] or genes involved in ER α signaling [18]. In cells undergoing osteogenic differentiation changes in the methylation status of the osteoblast-related gene Bglap2 (osteocalcin) gene were observed as a process of mechanical stimulation or differentiation [19, 20]. Treatment of MC-3T3-E1 osteoblasts with homocysteine in addition to IL-6 increased levels of DNMT1 and subsequent methylation at specific gene promoters [21]. In the same cell model system, osteoblasts plated on a collagen matrix or treated with DMSO demonstrated increased expression of DNMT1 and TET and an increase in global DNA methylation and methylation of the promoter of the apoptotic gene Fas [22, 23]. In a study involving and osteosarcoma model, a DNMT inhibitor reduced DNA methylation and increased expression of osteoblast-related genes such as IL-6, IL6ST, BMP7, ATP6B1, IGF1, WNT1, TNFs and ALPL [24].

Differentiation of osteoblasts from MSCs is an integral part of bone development and homeostasis and as such, the role of DNMTs in maintaining MSC quiescence, senescence and self-renewal has been studied. DNMT1 knockdown in early-passage MSCs induces senescence and reduces differentiation potential through a pRB-mediated pathway, whereas DNMT1 overexpression in late-passage MSCs prevents senescence [25]. Other studies demonstrated that the inhibition of DNMT1 in MSCs prevents differentiation to osteoblasts, adipocytes or chondrocytes by inducing replicative senescence mediated by p16 and p21 expression [26].

Histone post-translational modifications

Eukaryotic chromatin assembled into nucleosomes, which are complexes of multiple histone subunits and DNA that act both as physical barrier to DNA access and a source of epigenetic information. Post-translational modifications of histones proteins are a key component of epigenetic regulation influencing lineage commitment and gene expression. Reversible covalent modifications of histones occur at chemically labile residues (i.e. lysine, arginine,

serine, threonine, tyrosine and histidine) on accessible N- and C-terminal tail regions and in the less accessible histone fold or globular domains in the nucleosome core [27, 28]. Each specific modified residue on the histone protein may relay a specific piece of information. For example, methylation of lysine 4 of histone 3 (H3K4me3), which is a well-studied histone modification, generally indicates that a gene is transcriptionally-poised or active. This information is conferred through multiple mechanisms including altered histone protein-protein or histone protein-DNA interactions or recruitment of binding factors that affect histone modifying and remodeling activities [29]. The most commonly modified histone methylation sites include lysines; H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20 and arginines (R); H3R2, H3R8, H3R17, H3R26 and H4R3. However, several other residues throughout histone H1 and the core histone proteins H2A, H2B, H3 and H4 are modified in various contexts [30]. Although a specific histone modification can act individually and provide information on the expression state of a particular gene, the total of all modifications in a cell represent a comprehensive histone code relating the epigenetic state of a particular cell. This code becomes particularly complex as particular modifications may act synergistically or antagonistically and the extent or type of modification may vary at individual nucleosomes or individual cells in a population [5]. For example, many promoters in embryonic stem cells are marked by both an activating H3K4me3 mark and a repressive H3K27me3 mark which would appear conflicting with respect to simple models in which these marks are linked to, respectively, active and repressed transcriptional states [31]. A large number of histone-modifying enzymes are found in multi-subunit complexes and nearby, modified residues can create requisite binding sites for the components of the complex helping to anchor an enzyme to a nucleosome. For example, the COMPASS-like histone-modifying complexes can recognize and demethylate H3K27me3 (through the actions of UTX) while simultaneously methylating H3K4 (through the actions of MLL3/4) and acetylating H3K9 and H4K16 (through WDR5) [32].

Regulation of histone methylation and acetylation

Histone marks are regulated by proteins (or protein complexes) that incorporate (write) or remove (erase) specific covalent modifications and can be recognized by proteins that bind (read) specific modifications leading to altered gene expression (Figure 1). These proteins can be loosely characterized on their enzyme activity (e.g. histone/lysine deacetylase (HDAC/KDAC), histone/lysine acetyltransferase (HAT/KAT), lysine demethylase (KDM), or binding activity (e.g. bromodomain: proteins that bind acetylated lysines or chromodomain: proteins that bind methylated lysines). Three main families of enzymes have been identified thus far that catalyze the addition of methyl groups to lysine residues of histones (KMTs): the SET-domain-containing proteins, DOT1-like proteins, and protein arginine *N*-methyltransferases (PRMT) [30]. Enzymes that reverse the methylation of lysine residues (demethylases/KDMs) can be grouped into two basic families: amine oxidases and jumonji C (JmjC)-domain-containing, iron-dependent dioxygenases [30]. These enzymes are highly conserved from yeast to humans and are able to demethylate histone and non-histone substrates.

Acetylation of lysine residues entails the addition of an acetyl group on histone residues by lysine acetyltransferases (KATs) (sometimes referred to as histone acetyltransferases

(HATs)) and their removal by histone deacetylases (HDACs). There are two major classes of KATs based on homology to enzymes discovered in other model systems: the GNAT (Gcn5) family and the MYST (Moz/Ybf2/Sas2/Tip60) family. Other enzymes with less clearly conserved catalytic domains (such as CBP/p300 (KAT3A/KAT3B)) are functionally important in higher eukaryotes [33]. There are four classes of HDAC: classes I and II are closely related to yeast scRpd3 and scHda1, respectively, class III (referred to as sirtuins) are NAD⁺-dependent analogs to the yeast Sir2 protein, while class IV has only a single member (HDAC11) [34]. In addition to histone proteins many KATs and HDACs can act on a wide range of proteins both in the cytosol and the nucleus, extending their functional significance not only to epigenetic events but also cellular processes [35].

Histone modifications in bone

The histone code or epigenetic events leading to bone formation are relatively uncharacterized, however two recent studies have evaluated global histone modifications in the context of Runx2 binding in human MSCs and MC-3T3-E1 preosteoblasts during osteogenesis [36, 37]. In the study by Håkelién and colleagues, dynamic changes of several different histone methylations and acetylations were evaluated at two different osteogenic time points [36].

While the basic epigenetic modifications leading to bone formation are somewhat under studied, a significant amount of research has defined the regulatory roles of several histone modifiers in relation to bone formation (Table 1). The functions of several methyltransferases and demethylases have been directly evaluated in osteoblast or MSC models. Ito and colleagues determined through a complementary deletion/transgenic genetic approach, that the arginine methyltransferase PRMT4 (CARM1) is involved in endochondral ossification and chondrocyte proliferation through methylation of Sox9 [38]. However, chromatin targets were not tested in this study. The WHSC1 gene was originally identified due to its role in the human disease Wolf-Hirschhorn syndrome, a syndrome associated with bone abnormalities including craniofacial defects and growth abnormalities [39]. WHSC1 (also known as NSD2) encodes for a KMT that methylates H3K36 and deletion of this gene has severe effects on bone formation in mouse models through interactions with Runx2 and p300 [40]. In addition the related protein NSD1 also a H3K36-specific methyltransferase is associated with Soto syndrome, a condition characterized by macrocephaly and advanced bone ageing [41]. Several reports have suggested that the PRC2-associated H3K27 methyltransferase Ezh2 (KMT6) directly inhibits osteoblastogenesis either by promoting adipogenesis or blocking expression of phenotypic bone genes [42-44]. However it would appear that Ezh2 is required for osteochondrogenic progenitor establishment during differentiation from neural-crest cells [45]. Stimulation of MSCs with Wnt5a can induce the phosphorylation of histone methyltransferase, SETDB1 (KMT1E) leading to histone H3K9 methylation repressing PPAR- γ expression and adipogenesis thereby inducing osteogenesis [46].

Several studies have evaluated the role of demethylases in osteoblast commitment and differentiation. The PRC2-associated H3K27 methyltransferase Ezh2 (KMT6) directly inhibits osteoblastogenesis either by promoting adipogenesis or blocking expression of

phenotypic bone genes [42-44]. In contrast, expression of the H3K27 demethylase UTX (KDM6A) during mesenchymal commitment promotes osteogenesis over adipogenesis [44]. Decreased expression of *Imjd3* (KDM6B) or *JMJD2B* (KDM4B) resulted in increases H3K27 or H3K9 methylation (respectively) thereby suppressing osteoblast differentiation and reducing expression of several bone-related genes [47, 48]. The histone demethylase NO66 regulates the expression of several bone-related genes including *Osx* through active demethylation of H3K4 and H3K36, resulting in impaired osteogenesis [49, 50]. Bone-related signaling molecules can also induce epigenetic modifications. Stimulation of MSCs with *Wnt5a* increases the phosphorylation of histone methyltransferase, SETDB1 (KMT1E), leading to histone H3K9 methylation and repression of PPAR-gamma expression, thereby suppressing adipogenesis and inducing osteogenesis [46].

Several acetyltransferases have been demonstrated to be important to osteogenesis or bone formation. Mutations in the gene encoding CREB binding protein (CBP/KAT3A), a non-specific histone acetyltransferase are associated with Rubinstein-Taybi syndrome, an autosomal dominant disorder characterized by face and limb anomalies [51-53]. CBP has been also implicated as a direct- or co- regulator of several bone-related genes through acetylation of histone residues [54-57]. A frequent co-factor of CBP, p300 also functions as a general histone acetyltransferase and like CBP has been implicated in the acetylation and activation of bone-related gene promoters during osteogenesis [58-61]. WDR5 (WD repeat domain 5) is a component of the multi-subunit SET1 complex comprised of several histone-modifying proteins. WDR5 itself has both H3K4-specific methyltransferase and H4K8-specific acetyltransferase activity [62]. Transgenic overexpression of *Wdr5* using a 2.3-kb $\alpha(1)$ I-collagen promoter induced accelerated osteoblast and chondrocyte differentiation resulting in substantially increased endochondral bone growth suggesting [63]. Several other acetyltransferases have been implicated in epigenetic regulation of bone formation, osteoblast-related gene expression or deregulated in osteoarthritis including PCAF (KAT2B) [60, 64], MOZ/MORF (KAT6A/KAT6B) [65, 66], and the circadian-rhythm specific acetyltransferase: Clock (KAT13D) [67].

Amongst the histone-modifying enzymes, the most widely researched in the context of bone are the HDACs. Several studies have defined roles for several specific HDACs in regulating bone-related genes, in turn affecting osteoblast differentiation [68-73] (and reviewed in [74]). In addition, modulation of HDAC function can modulate extracellular signaling pathways, thereby affecting osteogenic commitment and differentiation in a context-dependent manner [75, 76]. A complicating factor with analysis of HDAC function is that like many acetyltransferases, HDACs can act on acetylated lysines of non-histone proteins. Many HDACs can deacetylate multiple cellular proteins, including the bone-related transcription factor *Runx2*, regulating their function through removal of acetylation or direct physical interaction [72, 77, 78]. As most HDAC proteins have no intrinsic chromatin binding ability, they are frequently associated with chromatin as transcriptional co-repressors. Several bone-related transcriptional regulators have been shown to be complexed with specific HDACs to control osteoblast-related gene expression in a context dependent manner including; *Runx2*, *NFATc1*, *Zfp521* and *Pbx1* [58, 78-80]. In addition to their direct

involvement in osteoblastogenesis, HDACs have also been demonstrated to contribute to osteoarthritis [81, 82]

Clinical relevance/mouse models of deregulated histone modifications

Drugs that alter the epigenome are entering the clinic at an unprecedented rate [83]. While hematopoietic and solid tumors are often the subject of clinical trials with these drugs, other conditions, including various forms of arthritis, diabetes, epilepsy, HIV infection, and neurodegeneration, are also being treated with epigenome-targeted therapies [84]. The first class of epigenetic drugs inhibited DNA methylation. Subsequently histone post-translational modifications were targeted with inhibitors of HDACs, KATs, KMTs, and KDMs. Histone deacetylase inhibitors (HDIs) are the most studied at this time, but drugs targeting histone methyltransferases (DotL1 and Ezh2) are now entered into clinical trials for cancer treatment. The mechanisms by which these drugs act are multifaceted and complex with both histone and non-histone proteins affected. However, it is broadly understood that changes in chromatin structure will alter gene expression and thus influence disease progression. Chromatin relaxation may also make cancerous cells more susceptible to DNA damage from radiation or chemotherapies.

The ubiquitous expression of histone modifiers raises the issue of specificity of the epigenetic drugs for diseased tissues and the risk of adverse events, especially for chronic diseases. Surprisingly, HDIs are fairly well tolerated, likely because they have short half-lives and their effects are reversible. The FDA-approved HDI, vorinostat, causes some generally mild problems, including thrombocytopenia, neutropenia, fatigue, diarrhea and dehydration, but all are temporary and cease after therapy ends [85]. A common feature of platelets, neutrophils and gastric epithelial cells is that they undergo continuous cellular regeneration. Bone is another tissue that is constantly remodeled and regenerated at both the tissue and cellular level. Considerable evidence suggests that long-term HDI use increases fracture risk and is teratogenic for fetuses [86, 87]. The assessment of animals treated with HDIs has produced a better understanding of how deregulated histone modifications affect the skeleton.

Several studies examined the effects of HDIs on bone tumor growth and bone density in animal models. Pratap and colleagues tested the ability of vorinostat to treat bone metastases of human breast and prostate cancer [88]. While daily HDI treatment (100 mg/kg, ip) for 4 weeks was effective at reducing tumor growth and osteolysis, it also caused significant cancellous bone loss in the contralateral non-tumor injected limbs. Because these studies required immunocompromised mice and micrometastases to the contralateral limbs could not be excluded, a parallel study was performed in tumor-free male C57Bl/6 mice. Using an identical treatment schedule, McGee-Lawrence and colleagues also observed severe cancellous bone loss in vorinostat-treated animals [89]. Cortical bone density was unaffected, as was bone resorption. Paradoxically, while osteoblast numbers were reduced in vorinostat treated bones, measures of osteoblast activity such as mineral apposition rate and bone formation rate were elevated. In vitro studies showed that immature osteoblasts were highly susceptible to DNA damage and cell cycle arrest when cultured in the presence of vorinostat [89, 90].

Although bone resorption was unaffected in the above study, there is a growing body of evidence that HDIs inhibit osteoclasts in animal models of chronic inflammatory diseases, including rheumatoid arthritis and periodontal disease [91]. A variety of HDIs suppress NF- κ B or NFAT signaling in osteoclasts and reduce expression of inflammatory cytokines, thereby slowing disease progression. Administration of HDIs via topical ointments or creams was effective in some studies and avoids systemic damage to the skeleton.

The observation that broad-acting HDIs can increase osteogenic activity of committed osteoblasts and other cells of mesenchymal origin has been independently reproduced in nearly a dozen laboratories around the world [92]. Thus, HDIs and perhaps other epigenetic drugs could be effectively used to engineer bone in vitro. However, the direct effects of the drugs on precursor cells remain a concern for in vivo bone regeneration [75]. Although some studies suggest that systemic administration of vorinostat at lower doses or reduced frequency may prevent the adverse skeletal effects of HDI therapy [90], finding an effective dose of a broad acting histone modifying drug that has no effects on a renewing tissue like bone, blood, or gastric epithelium may be impossible, just as it is for many cancer therapies, including chemotherapy and hormone-deprivation, and anti-inflammatory medications (e.g., corticosteroids). One way to address this problem is to gain a better understanding of which epigenetic modulators (readers, writers, and erasers) are expressed in bone cells so that more specific inhibitors can be designed for specific diseases.

Acknowledgements

This work was supported by NIAMS grants AR68103 (JJW), AR049069 (AJvW) and AR039588 and NIDCR grants DE20194 (JJW) and DE012528

References Cited

1. Mueller-Planitz F, Klinker H, Becker PB. Nucleosome sliding mechanisms: new twists in a looped history. *Nat Struct Mol Biol.* 2013; 20:1026–32. [PubMed: 24008565]
2. Kobayashi H, Kikyo N. Epigenetic regulation of open chromatin in pluripotent stem cells. *Transl Res.* 2015; 165:18–27. [PubMed: 24695097]
3. Miao CG, Yang YY, He X, Xu T, Huang C, Huang Y, Zhang L, Lv XW, Jin Y, Li J. New advances of microRNAs in the pathogenesis of rheumatoid arthritis, with a focus on the crosstalk between DNA methylation and the microRNA machinery. *Cell Signal.* 2013; 25:1118–25. [PubMed: 23385088]
4. Schaukowitch K, Kim TK. Emerging epigenetic mechanisms of long non-coding RNAs. *Neuroscience.* 2014; 264:25–38. [PubMed: 24342564]
5. Rothbart SB, Strahl BD. Interpreting the language of histone and DNA modifications. *Biochim Biophys Acta.* 2014; 1839:627–43. [PubMed: 24631868]
6. Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet.* 2010; 11:204–20. [PubMed: 20142834]
7. Lister R, Mukamel EA, Nery JR, Urich M, Puddifoot CA, Johnson ND, Lucero J, Huang Y, Dwork AJ, Schultz MD, Yu M, Tonti-Filippini J, Heyn H, Hu S, Wu JC, Rao A, Esteller M, He C, Haghghi FG, Sejnowski TJ, Behrens MM, Ecker JR. Global epigenomic reconfiguration during mammalian brain development. *Science.* 2013; 341:1237905. [PubMed: 23828890]
8. Shirane K, Toh H, Kobayashi H, Miura F, Chiba H, Ito T, Kono T, Sasaki H. Mouse oocyte methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. *PLoS Genet.* 2013; 9:e1003439. [PubMed: 23637617]

9. Wu H, Zhang Y. Reversing DNA methylation: mechanisms, genomics, and biological functions. *Cell*. 2014; 156:45–68. [PubMed: 24439369]
10. Deaton AM, Bird A. CpG islands and the regulation of transcription. *Genes Dev*. 2011; 25:1010–22. [PubMed: 21576262]
11. Payer B, Lee JT. X chromosome dosage compensation: how mammals keep the balance. *Annu Rev Genet*. 2008; 42:733–72. [PubMed: 18729722]
12. Smith ZD, Meissner A. DNA methylation: roles in mammalian development. *Nat Rev Genet*. 2013; 14:204–20. [PubMed: 23400093]
13. Long HK, Blackledge NP, Klose RJ. ZF-CxxC domain-containing proteins, CpG islands and the chromatin connection. *Biochem Soc Trans*. 2013; 41:727–40. [PubMed: 23697932]
14. Hashimoto K, Otero M, Imagawa K, de Andres MC, Coico JM, Roach HI, Oreffo RO, Marcu KB, Goldring MB. Regulated transcription of human matrix metalloproteinase 13 (MMP13) and interleukin-1beta (IL1B) genes in chondrocytes depends on methylation of specific proximal promoter CpG sites. *J Biol Chem*. 2013; 288:10061–72. [PubMed: 23417678]
15. Bui C, Barter MJ, Scott JL, Xu Y, Galler M, Reynard LN, Rowan AD, Young DA. cAMP response element-binding (CREB) recruitment following a specific CpG demethylation leads to the elevated expression of the matrix metalloproteinase 13 in human articular chondrocytes and osteoarthritis. *FASEB J*. 2012; 26:3000–11. [PubMed: 22505473]
16. de Andres MC, Imagawa K, Hashimoto K, Gonzalez A, Roach HI, Goldring MB, Oreffo RO. Loss of methylation in CpG sites in the NF-kappaB enhancer elements of inducible nitric oxide synthase is responsible for gene induction in human articular chondrocytes. *Arthritis Rheum*. 2013; 65:732–42. [PubMed: 23239081]
17. Dansranjav T, Krehl S, Mueller T, Mueller LP, Schmoll HJ, Dammann RH. The role of promoter CpG methylation in the epigenetic control of stem cell related genes during differentiation. *Cell Cycle*. 2009; 8:916–24. [PubMed: 19221495]
18. Hsiao SH, Lee KD, Hsu CC, Tseng MJ, Jin VX, Sun WS, Hung YC, Yeh KT, Yan PS, Lai YY, Sun HS, Lu YJ, Chang YS, Tsai SJ, Huang TH, Leu YW. DNA methylation of the Trip10 promoter accelerates mesenchymal stem cell lineage determination. *Biochem Biophys Res Commun*. 2010; 400:305–12. [PubMed: 20727853]
19. Arnsdorf EJ, Tummala P, Castillo AB, Zhang F, Jacobs CR. The epigenetic mechanism of mechanically induced osteogenic differentiation. *J Biomech*. 2010; 43:2881–6. [PubMed: 20728889]
20. Villagra A, Gutierrez J, Paredes R, Sierra J, Puchi M, Imschenetzky M, Wijnen Av A, Lian J, Stein G, Stein J, Montecino M. Reduced CpG methylation is associated with transcriptional activation of the bone-specific rat osteocalcin gene in osteoblasts. *J Cell Biochem*. 2002; 85:112–22. [PubMed: 11891855]
21. Thaler R, Agsten M, Spitzer S, Paschalis EP, Karlic H, Klaushofer K, Varga F. Homocysteine suppresses the expression of the collagen cross-linker lysyl oxidase involving IL-6, Fli1, and epigenetic DNA methylation. *J Biol Chem*. 2011; 286:5578–88. [PubMed: 21148317]
22. Thaler R, Karlic H, Spitzer S, Klaushofer K, Varga F. Extra-cellular matrix suppresses expression of the apoptosis mediator Fas by epigenetic DNA methylation. *Apoptosis*. 2010; 15:728–37. [PubMed: 20428952]
23. Thaler R, Spitzer S, Karlic H, Klaushofer K, Varga F. DMSO is a strong inducer of DNA hydroxymethylation in pre-osteoblastic MC3T3-E1 cells. *Epigenetics*. 2012; 7:635–51. [PubMed: 22507896]
24. Capobianco E, Mora A, La Sala D, Roberti A, Zaki N, Badidi E, Taranta M, Cinti C. Separate and combined effects of DNMT and HDAC inhibitors in treating human multi-drug resistant osteosarcoma HosDXR150 cell line. *PLoS One*. 2014; 9:e95596. [PubMed: 24756038]
25. Lin SP, Chiu FY, Wang Y, Yen ML, Kao SY, Hung SC. RB Maintains Quiescence and Prevents Premature Senescence through Upregulation of DNMT1 in Mesenchymal Stromal Cells. *Stem Cell Reports*. 2014
26. Tsai CC, Su PF, Huang YF, Yew TL, Hung SC. Oct4 and Nanog directly regulate Dnmt1 to maintain self-renewal and undifferentiated state in mesenchymal stem cells. *Mol Cell*. 2012; 47:169–82. [PubMed: 22795133]

27. Kouzarides T. Chromatin modifications and their function. *Cell*. 2007; 128:693–705. [PubMed: 17320507]
28. Huang H, Sabari BR, Garcia BA, Allis CD, Zhao Y. SnapShot: histone modifications. *Cell*. 2014; 159:458–458. e1. [PubMed: 25303536]
29. Lee JS, Smith E, Shilatifard A. The language of histone crosstalk. *Cell*. 2010; 142:682–5. [PubMed: 20813257]
30. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet*. 2012; 13:343–57. [PubMed: 22473383]
31. Voigt P, Tee WW, Reinberg D. A double take on bivalent promoters. *Genes Dev*. 2013; 27:1318–38. [PubMed: 23788621]
32. Schuettengruber B, Martinez AM, Iovino N, Cavalli G. Trithorax group proteins: switching genes on and keeping them active. *Nat Rev Mol Cell Biol*. 2011; 12:799–814. [PubMed: 22108599]
33. Lalonde ME, Cheng X, Cote J. Histone target selection within chromatin: an exemplary case of teamwork. *Genes Dev*. 2014; 28:1029–41. [PubMed: 24831698]
34. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011; 21:381–95. [PubMed: 21321607]
35. Choudhary C, Weinert BT, Nishida Y, Verdin E, Mann M. The growing landscape of lysine acetylation links metabolism and cell signalling. *Nat Rev Mol Cell Biol*. 2014; 15:536–50. [PubMed: 25053359]
36. Hakelien AM, Bryne JC, Harstad KG, Lorenz S, Paulsen J, Sun J, Mikkelsen TS, Myklebost O, Meza-Zepeda LA. The regulatory landscape of osteogenic differentiation. *Stem Cells*. 2014; 32:2780–93. [PubMed: 24898411]
37. Meyer MB, Benkuský NA, Pike JW. The RUNX2 cistrome in osteoblasts: characterization, down-regulation following differentiation, and relationship to gene expression. *J Biol Chem*. 2014; 289:16016–31. [PubMed: 24764292]
38. Ito T, Yadav N, Lee J, Furumatsu T, Yamashita S, Yoshida K, Taniguchi N, Hashimoto M, Tsuchiya M, Ozaki T, Lotz M, Bedford MT, Asahara H. Arginine methyltransferase CARM1/PRMT4 regulates endochondral ossification. *BMC Dev Biol*. 2009; 9:47. [PubMed: 19725955]
39. Nimura K, Ura K, Shiratori H, Ikawa M, Okabe M, Schwartz RJ, Kaneda Y. A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf-Hirschhorn syndrome. *Nature*. 2009; 460:287–91. [PubMed: 19483677]
40. Lee YF, Nimura K, Lo WN, Saga K, Kaneda Y. Histone H3 lysine 36 methyltransferase Whsc1 promotes the association of Runx2 and p300 in the activation of bone-related genes. *PLoS One*. 2014; 9:e106661. [PubMed: 25188294]
41. Leventopoulos G, Kitsiou-Tzeli S, Kritikos K, Psoni S, Mavrou A, Kanavakis E, Fryssira H. A clinical study of Sotos syndrome patients with review of the literature. *Pediatr Neurol*. 2009; 40:357–64. [PubMed: 19380072]
42. Wei Y, Chen YH, Li LY, Lang J, Yeh SP, Shi B, Yang CC, Yang JY, Lin CY, Lai CC, Hung MC. CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. *Nat Cell Biol*. 2011; 13:87–94. [PubMed: 21131960]
43. Zhu L, Xu PC. Downregulated LncRNA-ANCR promotes osteoblast differentiation by targeting EZH2 and regulating Runx2 expression. *Biochem Biophys Res Commun*. 2013; 432:612–7. [PubMed: 23438432]
44. Hemming S, Cakouros D, Isenmann S, Cooper L, Menicanin D, Zannettino A, Gronthos S. EZH2 and KDM6A act as an epigenetic switch to regulate mesenchymal stem cell lineage specification. *Stem Cells*. 2014; 32:802–15. [PubMed: 24123378]
45. Schwarz D, Varum S, Zemke M, Scholer A, Baggiolini A, Draganova K, Koseki H, Schubeler D, Sommer L. Ezh2 is required for neural crest-derived cartilage and bone formation. *Development*. 2014; 141:867–77. [PubMed: 24496623]
46. Takada I, Mihara M, Suzawa M, Ohtake F, Kobayashi S, Igarashi M, Youn MY, Takeyama K, Nakamura T, Mezaki Y, Takezawa S, Yogiashi Y, Kitagawa H, Yamada G, Takada S, Minami Y, Shibuya H, Matsumoto K, Kato S. A histone lysine methyltransferase activated by non-canonical

- Wnt signalling suppresses PPAR-gamma transactivation. *Nat Cell Biol.* 2007; 9:1273–85. [PubMed: 17952062]
47. Yang D, Okamura H, Nakashima Y, Haneji T. Histone demethylase Jmjd3 regulates osteoblast differentiation via transcription factors Runx2 and osterix. *J Biol Chem.* 2013; 288:33530–41. [PubMed: 24106268]
 48. Ye L, Fan Z, Yu B, Chang J, Al Hezaimi K, Zhou X, Park NH, Wang CY. Histone demethylases KDM4B and KDM6B promotes osteogenic differentiation of human MSCs. *Cell Stem Cell.* 2012; 11:50–61. [PubMed: 22770241]
 49. Sinha KM, Yasuda H, Coombes MM, Dent SY, de Crombrughe B. Regulation of the osteoblast-specific transcription factor Osterix by NO66, a Jumonji family histone demethylase. *EMBO J.* 2010; 29:68–79. [PubMed: 19927124]
 50. Sinha KM, Yasuda H, Zhou X, deCrombrughe B. Osterix and NO66 histone demethylase control the chromatin of Osterix target genes during osteoblast differentiation. *J Bone Miner Res.* 2014; 29:855–65. [PubMed: 24115157]
 51. Tanaka Y, Naruse I, Maekawa T, Masuya H, Shiroishi T, Ishii S. Abnormal skeletal patterning in embryos lacking a single Cbp allele: a partial similarity with Rubinstein-Taybi syndrome. *Proc Natl Acad Sci U S A.* 1997; 94:10215–20. [PubMed: 9294190]
 52. Allanson JE, Hennekam RC. Rubinstein-Taybi syndrome: objective evaluation of craniofacial structure. *Am J Med Genet.* 1997; 71:414–9. [PubMed: 9286447]
 53. Park E, Kim Y, Ryu H, Kowall NW, Lee J, Ryu H. Epigenetic mechanisms of Rubinstein-Taybi syndrome. *Neuromolecular Med.* 2014; 16:16–24. [PubMed: 24381114]
 54. Gordon JA, Hassan MQ, Saini S, Montecino M, van Wijnen AJ, Stein GS, Stein JL, Lian JB. Pbx1 represses osteoblastogenesis by blocking Hoxa10-mediated recruitment of chromatin remodeling factors. *Mol Cell Biol.* 2010; 30:3531–41. [PubMed: 20439491]
 55. Greenblatt MB, Shim JH, Zou W, Sitara D, Schweitzer M, Hu D, Lotinun S, Sano Y, Baron R, Park JM, Arthur S, Xie M, Schneider MD, Zhai B, Gygi S, Davis R, Glimcher LH. The p38 MAPK pathway is essential for skeletogenesis and bone homeostasis in mice. *J Clin Invest.* 2010; 120:2457–73. [PubMed: 20551513]
 56. Kim S, Shevde NK, Pike JW. 1,25-Dihydroxyvitamin D3 stimulates cyclic vitamin D receptor/retinoid X receptor DNA-binding, co-activator recruitment, and histone acetylation in intact osteoblasts. *J Bone Miner Res.* 2005; 20:305–17. [PubMed: 15647825]
 57. Yang D, Guo J, Divieti P, Shioda T, Bringhurst FR. CBP/p300-interacting protein CITED1 modulates parathyroid hormone regulation of osteoblastic differentiation. *Endocrinology.* 2008; 149:1728–35. [PubMed: 18187554]
 58. Gordon JA, Hassan MQ, Koss M, Montecino M, Selleri L, van Wijnen AJ, Stein JL, Stein GS, Lian JB. Epigenetic regulation of early osteogenesis and mineralized tissue formation by a HOXA10-PBX1-associated complex. *Cells Tissues Organs.* 2011; 194:146–50. [PubMed: 21597276]
 59. Jun JH, Yoon WJ, Seo SB, Woo KM, Kim GS, Ryoo HM, Baek JH. BMP2-activated Erk/MAP kinase stabilizes Runx2 by increasing p300 levels and histone acetyltransferase activity. *J Biol Chem.* 2010; 285:36410–9. [PubMed: 20851880]
 60. Lee M, Partridge NC. Parathyroid hormone activation of matrix metalloproteinase-13 transcription requires the histone acetyltransferase activity of p300 and PCAF and p300-dependent acetylation of PCAF. *J Biol Chem.* 2010; 285:38014–22. [PubMed: 20870727]
 61. Rodriguez-Carballo E, Ulsamer A, Susperregui AR, Manzanares-Céspedes C, Sanchez-Garcia E, Bartrons R, Rosa JL, Ventura F. Conserved regulatory motifs in osteogenic gene promoters integrate cooperative effects of canonical Wnt and BMP pathways. *J Bone Miner Res.* 2011; 26:718–29. [PubMed: 20878775]
 62. Migliori V, Mapelli M, Guccione E. On WD40 proteins: propelling our knowledge of transcriptional control? *Epigenetics.* 2012; 7:815–22. [PubMed: 22810296]
 63. Gori F, Friedman LG, Demay MB. Wdr5, a WD-40 protein, regulates osteoblast differentiation during embryonic bone development. *Dev Biol.* 2006; 295:498–506. [PubMed: 16730692]

64. Wang CY, Yang SF, Wang Z, Tan JM, Xing SM, Chen DC, Xu SM, Yuan W. PCAF acetylates Runx2 and promotes osteoblast differentiation. *J Bone Miner Metab.* 2013; 31:381–9. [PubMed: 23468178]
65. Campeau PM, Kim JC, Lu JT, Schwartzenruber JA, Abdul-Rahman OA, Schlaubitz S, Murdock DM, Jiang MM, Lammer EJ, Enns GM, Rhead WJ, Rowland J, Robertson SP, Cormier-Daire V, Bainbridge MN, Yang XJ, Gingras MC, Gibbs RA, Rosenblatt DS, Majewski J, Lee BH. Mutations in KAT6B, encoding a histone acetyltransferase, cause Genitopatellar syndrome. *Am J Hum Genet.* 2012; 90:282–9. [PubMed: 22265014]
66. Pelletier N, Champagne N, Stifani S, Yang XJ. MOZ and MORF histone acetyltransferases interact with the Runt-domain transcription factor Runx2. *Oncogene.* 2002; 21:2729–40. [PubMed: 11965546]
67. Kanbe K, Inoue K, Xiang C, Chen Q. Identification of clock as a mechanosensitive gene by large-scale DNA microarray analysis: downregulation in osteoarthritic cartilage. *Mod Rheumatol.* 2006; 16:131–6. [PubMed: 16767550]
68. Bradley EW, Carpio LR, Olson EN, Westendorf JJ. Histone Deacetylase 7 (Hdac7) Suppresses Chondrocyte Proliferation and beta-Catenin Activity During Endochondral Ossification. *J Biol Chem.* 2014
69. Fu Y, Zhang P, Ge J, Cheng J, Dong W, Yuan H, Du Y, Yang M, Sun R, Jiang H. Histone deacetylase 8 suppresses osteogenic differentiation of bone marrow stromal cells by inhibiting histone H3K9 acetylation and RUNX2 activity. *Int J Biochem Cell Biol.* 2014; 54:68–77. [PubMed: 25019367]
70. Lee HW, Suh JH, Kim AY, Lee YS, Park SY, Kim JB. Histone deacetylase 1-mediated histone modification regulates osteoblast differentiation. *Mol Endocrinol.* 2006; 20:2432–43. [PubMed: 16728531]
71. Paino F, La Noce M, Tirino V, Naddeo P, Desiderio V, Pirozzi G, De Rosa A, Laino L, Altucci L, Papaccio G. Histone deacetylase inhibition with valproic acid downregulates osteocalcin gene expression in human dental pulp stem cells and osteoblasts: evidence for HDAC2 involvement. *Stem Cells.* 2014; 32:279–89. [PubMed: 24105979]
72. Schroeder TM, Kahler RA, Li X, Westendorf JJ. Histone deacetylase 3 interacts with runx2 to repress the osteocalcin promoter and regulate osteoblast differentiation. *J Biol Chem.* 2004; 279:41998–2007. [PubMed: 15292260]
73. Shimizu E, Nakatani T, He Z, Partridge NC. Parathyroid hormone regulates histone deacetylase (HDAC) 4 through protein kinase A-mediated phosphorylation and dephosphorylation in osteoblastic cells. *J Biol Chem.* 2014; 289:21340–50. [PubMed: 24904057]
74. Bradley EW, McGee-Lawrence ME, Westendorf JJ. Hdac-mediated control of endochondral and intramembranous ossification. *Crit Rev Eukaryot Gene Expr.* 2011; 21:101–13. [PubMed: 22077150]
75. Dudakovic A, Camilleri ET, Lewallen EA, McGee-Lawrence ME, Riestter SM, Kakar S, Montecino M, Stein GS, Ryoo HM, Dietz AB, Westendorf JJ, van Wijnen AJ. Histone deacetylase inhibition destabilizes the multi-potent state of uncommitted adipose-derived mesenchymal stromal cells. *J Cell Physiol.* 2015; 230:52–62. [PubMed: 24912092]
76. Dudakovic A, Evans JM, Li Y, Middha S, McGee-Lawrence ME, van Wijnen AJ, Westendorf JJ. Histone deacetylase inhibition promotes osteoblast maturation by altering the histone H4 epigenome and reduces Akt phosphorylation. *J Biol Chem.* 2013; 288:28783–91. [PubMed: 23940046]
77. Pham L, Kaiser B, Romsa A, Schwarz T, Gopalakrishnan R, Jensen ED, Mansky KC. HDAC3 and HDAC7 have opposite effects on osteoclast differentiation. *J Biol Chem.* 2011; 286:12056–65. [PubMed: 21324898]
78. Jensen ED, Schroeder TM, Bailey J, Gopalakrishnan R, Westendorf JJ. Histone deacetylase 7 associates with Runx2 and represses its activity during osteoblast maturation in a deacetylation-independent manner. *J Bone Miner Res.* 2008; 23:361–72. [PubMed: 17997710]
79. Choo MK, Yeo H, Zayzafoon M. NFATc1 mediates HDAC-dependent transcriptional repression of osteocalcin expression during osteoblast differentiation. *Bone.* 2009; 45:579–89. [PubMed: 19463978]

80. Hesse E, Saito H, Kiviranta R, Correa D, Yamana K, Neff L, Toben D, Duda G, Atfi A, Geoffroy V, Horne WC, Baron R. Zfp521 controls bone mass by HDAC3-dependent attenuation of Runx2 activity. *J Cell Biol.* 2010; 191:1271–83. [PubMed: 21173110]
81. Chen WP, Bao JP, Hu PF, Feng J, Wu LD. Alleviation of osteoarthritis by Trichostatin A, a histone deacetylase inhibitor, in experimental osteoarthritis. *Mol Biol Rep.* 2010; 37:3967–72. [PubMed: 20237852]
82. Culley KL, Hui W, Barter MJ, Davidson RK, Swingle TE, Destrument AP, Scott JL, Donell ST, Fenwick S, Rowan AD, Young DA, Clark IM. Class I histone deacetylase inhibition modulates metalloproteinase expression and blocks cytokine-induced cartilage degradation. *Arthritis Rheum.* 2013; 65:1822–30. [PubMed: 23575963]
83. Boumber Y, Issa JP. Epigenetics in cancer: what's the future? *Oncology.* 2011; 25:220–6. 228. [PubMed: 21548464]
84. Dinarello CA, Fossati G, Mascagni P. Histone Deacetylase Inhibitors for Treating a Spectrum of Diseases Not Related to Cancer. *Mol Med.* 2011; 17:333–352. [PubMed: 21556484]
85. Marks PA. Discovery and development of SAHA as an anticancer agent. *Oncogene.* 2007; 26:1351–6. [PubMed: 17322921]
86. Senn SM, Kantor S, Poulton IJ, Morris MJ, Sims NA, O'Brien TJ, Wark JD. Adverse effects of valproate on bone: defining a model to investigate the pathophysiology. *Epilepsia.* 2010; 51:984–93. [PubMed: 20163440]
87. Giavini E, Menegola E. Teratogenic Activity of HDAC Inhibitors. *Current pharmaceutical design.* 2014
88. Pratap J, Akech J, Wixted JJ, Szabo G, Hassain S, McGee-Lawrence ME, Li X, Bedard K, Dhillon RJ, van Wijnen AJ, Stein JL, Stein GS, Westendorf JJ, Lian JB. The histone deacetylase inhibitor, vorinostat, reduces tumor growth at the metastatic bone site and associated osteolysis, but promotes normal bone loss. *Mol Cancer Ther.* 2010; 9:3210–20. [PubMed: 21159607]
89. McGee-Lawrence ME, McCleary-Wheeler AL, Secreto FJ, Razidlo DF, Zhang M, Stensgard BA, Li X, Stein GS, Lian JB, Westendorf JJ. Suberoylanilide hydroxamic acid (SAHA; vorinostat) causes bone loss by inhibiting immature osteoblasts. *Bone.* 2011; 48:1117–26. [PubMed: 21255693]
90. Xu S, De Veirman K, Evans H, Santini GC, Vande Broek I, Leleu X, De Becker A, Van Camp B, Croucher P, Vanderkerken K, Van Riet I. Effect of the HDAC inhibitor vorinostat on the osteogenic differentiation of mesenchymal stem cells in vitro and bone formation in vivo. *Acta pharmacologica Sinica.* 2013; 34:699–709. [PubMed: 23564084]
91. Cantley MD, Bartold PM, Fairlie DP, Rainsford KD, Haynes DR. Histone deacetylase inhibitors as suppressors of bone destruction in inflammatory diseases. *The Journal of pharmacy and pharmacology.* 2012; 64:763–74. [PubMed: 22571254]
92. McGee-Lawrence ME, Westendorf JJ. Histone deacetylases in skeletal development and bone mass maintenance. *Gene.* 2011; 474:1–11. [PubMed: 21185361]
93. Yamada-Okabe T, Imamura K, Kawaguchi N, Sakai H, Yamashita M, Matsumoto N. Functional characterization of the zebrafish WHSC1-related gene, a homolog of human NSD2. *Biochem Biophys Res Commun.* 2010; 402:335–9. [PubMed: 20946879]
94. Mendelsohn BA, Pronold M, Long R, Smaoui N, Slavotinek AM. Advanced bone age in a girl with Wiedemann-Steiner syndrome and an exonic deletion in KMT2A (MLL). *Am J Med Genet A.* 2014; 164A:2079–83. [PubMed: 24818805]
95. Oppenheimer H, Kumar A, Meir H, Schwartz I, Zini A, Haze A, Kandel L, Mattan Y, Liebergall M, Dvir-Ginzberg M. Set7/9 impacts COL2A1 expression through binding and repression of SirT1 histone deacetylation. *J Bone Miner Res.* 2014; 29:348–60. [PubMed: 23873758]
96. Rodova M, Lu Q, Li Y, Woodbury BG, Crist JD, Gardner BM, Yost JG, Zhong XB, Anderson HC, Wang J. Nfat1 regulates adult articular chondrocyte function through its age-dependent expression mediated by epigenetic histone methylation. *J Bone Miner Res.* 2011; 26:1974–86. [PubMed: 21452283]
97. Gori F, Zhu ED, Demay MB. Perichondrial expression of Wdr5 regulates chondrocyte proliferation and differentiation. *Dev Biol.* 2009; 329:36–43. [PubMed: 19217897]

98. Zhao QH, Wang SG, Liu SX, Li JP, Zhang YX, Sun ZY, Fan QM, Tian JW. PPARgamma forms a bridge between DNA methylation and histone acetylation at the C/EBPalpha gene promoter to regulate the balance between osteogenesis and adipogenesis of bone marrow stromal cells. *FEBS J.* 2013; 280:5801–14. [PubMed: 23981481]
99. Singh N, Gupta M, Trivedi CM, Singh MK, Li L, Epstein JA. Murine craniofacial development requires Hdac3-mediated repression of Msx gene expression. *Dev Biol.* 2013; 377:333–44. [PubMed: 23506836]
100. McGee-Lawrence ME, Bradley EW, Dudakovic A, Carlson SW, Ryan ZC, Kumar R, Dadsetan M, Yaszemski MJ, Chen Q, An KN, Westendorf JJ. Histone deacetylase 3 is required for maintenance of bone mass during aging. *Bone.* 2013; 52:296–307. [PubMed: 23085085]
101. Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X, Meadows E, McAnally J, Pomajzl C, Shelton JM, Richardson JA, Karsenty G, Olson EN. Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. *Cell.* 2004; 119:555–66. [PubMed: 15537544]
102. Sun X, Wei L, Chen Q, Terek RM. HDAC4 represses vascular endothelial growth factor expression in chondrosarcoma by modulating RUNX2 activity. *J Biol Chem.* 2009; 284:21881–90. [PubMed: 19509297]
103. Obri A, Makinistoglu MP, Zhang H, Karsenty G. HDAC4 integrates PTH and sympathetic signaling in osteoblasts. *J Cell Biol.* 2014; 205:771–80. [PubMed: 24934156]
104. Wein MN, Spatz J, Nishimori S, Doench J, Root D, Babij P, Nagano K, Baron R, Brooks D, Bouxsein M, Pajevic PD, Kronenberg HM. HDAC5 controls MEF2C-driven sclerostin expression in osteocytes. *J Bone Miner Res.* 2014
105. Jeon EJ, Lee KY, Choi NS, Lee MH, Kim HN, Jin YH, Ryoo HM, Choi JY, Yoshida M, Nishino N, Oh BC, Lee KS, Lee YH, Bae SC. Bone morphogenetic protein-2 stimulates Runx2 acetylation. *J Biol Chem.* 2006; 281:16502–11. [PubMed: 16613856]
106. Westendorf JJ, Zaidi SK, Cascino JE, Kahler R, van Wijnen AJ, Lian JB, Yoshida M, Stein GS, Li X. Runx2 (Cbfa1, AML-3) interacts with histone deacetylase 6 and represses the p21(CIP1/WAF1) promoter. *Mol Cell Biol.* 2002; 22:7982–92. [PubMed: 12391164]
107. Jensen ED, Gopalakrishnan R, Westendorf JJ. Bone morphogenic protein 2 activates protein kinase D to regulate histone deacetylase 7 localization and repression of Runx2. *J Biol Chem.* 2009; 284:2225–34. [PubMed: 19029091]
108. Haberland M, Mokalled MH, Montgomery RL, Olson EN. Epigenetic control of skull morphogenesis by histone deacetylase 8. *Genes Dev.* 2009; 23:1625–30. [PubMed: 19605684]
109. Yoon DS, Choi Y, Jang Y, Lee M, Choi WJ, Kim SH, Lee JW. SIRT1 Directly Regulates SOX2 to Maintain Self-Renewal and Multipotency in Bone Marrow-Derived Mesenchymal Stem Cells. *Stem Cells.* 2014; 32:3219–31. [PubMed: 25132403]
110. Gabay O, Zaal KJ, Sanchez C, Dvir-Ginzberg M, Gagarina V, Song Y, He XH, McBurney MW. Sirt1-deficient mice exhibit an altered cartilage phenotype. *Joint Bone Spine.* 2013; 80:613–20. [PubMed: 23587642]
111. Simic P, Zainabadi K, Bell E, Sykes DB, Saez B, Lotinun S, Baron R, Scadden D, Schipani E, Guarente L. SIRT1 regulates differentiation of mesenchymal stem cells by deacetylating beta-catenin. *EMBO Mol Med.* 2013; 5:430–40. [PubMed: 23364955]
112. Cohen-Kfir E, Artsi H, Levin A, Abramowitz E, Bajayo A, Gurt I, Zhong L, D'Urso A, Toiber D, Mostoslavsky R, Dresner-Pollak R. Sirt1 is a regulator of bone mass and a repressor of Sost encoding for sclerostin, a bone formation inhibitor. *Endocrinology.* 2011; 152:4514–24. [PubMed: 21952235]
113. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, Mills KD, Patel P, Hsu JT, Hong AL, Ford E, Cheng HL, Kennedy C, Nunez N, Bronson R, Frendewey D, Auerbach W, Valenzuela D, Karow M, Hottiger MO, Hursting S, Barrett JC, Guarente L, Mulligan R, Demple B, Yancopoulos GD, Alt FW. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell.* 2006; 124:315–29. [PubMed: 16439206]
114. Piao J, Tsuji K, Ochi H, Iwata M, Koga D, Okawa A, Morita S, Takeda S, Asou Y. Sirt6 regulates postnatal growth plate differentiation and proliferation via Ihh signaling. *Sci Rep.* 2013; 3:3022. [PubMed: 24149372]

Transcriptionally inactive chromatin

Transcriptionally active chromatin

- histone methylation ("permissive") ■ DNA methylation
- histone methylation ("repressive") ● acetylation

Figure 1. Regulation of gene expression by chromatin modifiers

Schematic overview of activity of chromatin modifiers regulating gene expression.

Opposing actions of lysine methyltransferases (KMTs) and lysine demethylases (KDMs) serve to add or remove methyl groups to histone proteins of chromatin, respectively. DNA methyltransferase (DNMT) activity results in transfer to methyl groups to cytosine on chromatin-associated DNA. Removal or addition of acetyl residues is facilitated by histone deacetylases (HDACs) or lysine acetyltransferases (KATs), respectively.

Table 1

Chromatin modifying enzymes involved in bone formation or regulation of bone genes

Enzyme	Alias	Histone residue targets	Role in bone formation	Reference
Methyltransferases				
CARM1	PRMT4	H3R2; H3R17; H3R26	Endochondral bone formation	[38]
WHSC1	NSD2	H3K36	Regulation of bone genes; developmental bone formation	[40, 93]
NSD1	KMT3B	H3K36	Regulation of bone genes; Advanced bone age; Soto syndrome	[41]
MLL1	KMT2A	H3K4	Advanced bone age	[94]
SETD7	SET7/9, KMT7	H3K4	Regulation of chondrocyte-related genes in chondrocytes	[95]
EZH2	KMT6	H3K27	Required for progenitor formation; inhibits osteogenesis	[42-45]
SETDB1	KMT1E	H3K9	Suppression of adipogenesis in progenitors	[46]
Demethylases				
KDM1A	LSD1, AOF2	H3K4; H3K9	Regulation of Nfat1-mediated chondrogenesis	[96]
KDM4B	JMJD2B	H3K9; H3K36	Promotes osteogenic differentiation	[48]
KDM6A	UTX	H3K27	Inhibits osteogenesis	[44]
KDM6B	JMJD3	H3K27	Promotes osteogenic differentiation	[48]
C14orf169	NO66	H3K4; H3K36	Inhibits osteogenesis	[49, 50]
Acetyltransferases				
KAT2B	PCAF	H2B; H3	Regulation of bone-related genes	[60, 64]
CREBBP				
	CBP, KAT3A	H2A; H2B; H3	Co-regulation of bone genes; Rubenstein-Taybi syndrome	[51-57]
EP300	KAT3B	H3	Co-regulation of bone-related genes	[58-61]
KAT6A	MOZ, MYST3, RUNXBP2	H3; H4	Bone formation in Genitopatellar syndrome; Interaction with Runx2	[65, 66]
KAT6B	MORF, MYST4	H3; H4	Interaction with Runx2	[66]
WDR5	BIG-3, SWD3	H3K4; H4K8	Developmental bone formation	[63, 97]
Deacetylases				
HDAC1	Histone Deacetylase 1; KDAC1	H2A; H2B; H3; H4	Transcriptional co-regulator; Regulation of osteoblast differentiation	[70, 98]
HDAC2	Histone Deacetylase 2	H2A; H2B; H3; H4	Regulation of osteoblast differentiation	[71]
HDAC3	Histone Deacetylase 3	H2A; H2B; H3; H3K27; H4	Craniofacial development; Maintenance of bone mass during aging; Negative regulator of osteoblast gene expression	[72, 99, 100]
HDAC4	Histone Deacetylase 4	H2A; H2B; H3; H4	Transcriptional co-regulator; Regulator of chondrocyte hypertrophy and skeletogenesis; Modulation of Runx2 activity	[73, 101-103]
HDAC5	Histone Deacetylase 5	H2A; H2B; H3; H4	Negative regulation of osteoblast gene expression; Modulation of Runx2 activity	[104, 105]

Enzyme	Alias	Histone residue targets	Role in bone formation	Reference
HDAC6	Histone Deacetylase 6; Tdac	H2A; H2B: H3; H4	Modulation of Runx2 activity; Regulation of gene expression	[106]
HDAC7	Histone Deacetylase 7	H2A; H2B: H3; H4	Regulation of chondrocyte hypertrophy; Negative regulation of osteoblast gene expression; Modulation of transcriptional activators	[68, 77, 107]
HDAC8	Histone Deacetylase 8	H2A; H2B: H3; H3K9; H4	Negative regulation of osteoblast differentiation; Craniofacial development	[69, 108]
SIRT1	SIR2L1			
	H3; H4	MSC renewal; Regulation of cartilage and bone formation; Regulation of osteoblast differentiation	[109-112]	
SIRT6	SIR2L6			
	H3; H4; H3K9; H3K56			
	Osteoporosis and reduced bone mineral density in null-animals; Regulation of chondrocyte proliferation and hypertrophy	[113, 114]		