Emergence of the Osteo-Epigenome in Bone Biology

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Abstract

Bone development and bone remodeling are accompanied by bone cell-specific gene regulation. Recent progress in gene expression has revealed that chromatin remodeling and histone modifications are indispensable for transcriptional activation as well as repression. Previously known transcriptional co-regulators have consistently been found to regulate chromatin reorganization, constituting an epigenetic platform on chromatin. As in other tested cells, bone cell type-specific chromatin reorganization is thought to mediate the function of prime transcriptional factors, which are responsible for bone cell fate decision and cellular differentiation. In this Perspective, we review chromatin reorganization and its regulators in bone cells, along with recent pioneering work describing osteo-epigenetic regulators.

The Transcriptional Network in Bone Development and Remodeling

From its first appearance in embryos, bone's formation and maintenance is a dynamic and complex process. Complicated but highly regulated differentiation steps guide the generation of bone tissue, processes that continue until full growth is achieved. In adulthood, even though net increases in bone have ceased, bone remodeling remains active to keep serum mineral levels physiologically normal in response to demands, and to maintain a physically solid bone structure against mechanical stress. In this respect, bone cells are like other cell types as their proliferation and differentiation are highly regulated through the entire life of the animal (1). Osteoblasts, osteocytes and chondrocytes originate from mesenchymal stem cells, whereas osteoclast precursors arise from hematopoietic stem cells (2-5). Autonomous cell proliferation and differentiation of these bone cells are regulated in a bone cell type-specific manner but “cross-talk” among different types of bone cells is significant during bone tissue development and bone remodeling (6,7).

Reflecting the complexity of the proliferation and differentiation of bone cells, a number of regulators have been identified and their physiological impact in intact bone has been confirmed by a variety of experimental approaches (1-8). Among such osteo-regulators, transcriptional regulators appear pivotal in cell lineage decisions (9-11). Transcriptional controls at a given type II gene locus require in general three classes of transcription factors. Basic transcription factors are essential for a transcriptional reaction by RNA polymerase II. Assembly of a set of basic transcription factors assures the minimum conditions for initiation of transcription (12). The enhancement or suppression of transcription depends on the participation of DNA-binding transcription factors. Since DNA-binding transcription factors direct transcriptional regulation, the expression and function of this class of transcription factors are prime determinants in tissue-specific gene cascades. These two classes of transcription factors are emphasized in the current literature from a classical point of view. However, a third class of factors, transcriptional co-regulators, has emerged and is now understood to be essential for transcriptional control (13) (Fig. 1). The role of these co-
regulators in transcriptional control had been unclear except in the case of overt coregulation of DNA-binding transcription factors in certain artificial experimental settings such as luciferase reporter assays in vitro. However, recent progress in the characterization of co-regulators has revealed that their major functions are associated with chromatin reorganization and histone modifications, and they often form functional units as multicomponent complexes (14-20).

**Fig. 1. Transcriptional controls exerted by three classes of transcription factors in gene promoters.** Transcription factors consist of three classes: basic transcription factors, DNA-binding transcription factors and transcriptional co-regulators. A minimal set of basic transcription factors is indispensable for transcriptional initiation in all the gene promoters, while DNA-binding transcription factors are recruited only when their specific DNA-binding motifs are present in the promoters. The roles of transcriptional co-regulators largely remain to be uncovered.

**Chromatin Reorganization Is a Prerequisite for Transcriptional Control**

Nucleosomal units are in general inhibitory for transcriptional events because the physical interaction between chromosomal DNA and histone octamers hinders local DNA sequences from associating with DNA-binding transcription factors (21). Therefore, transcriptional events directed by DNA-binding transcription factors require chromatin reorganization to enable the factors to specifically recognize and stably bind to specific DNA sequences. The canonical histone octamer is composed of H2A, H2B, H3 and H4 (22). However, recent studies of histone protein variants have revealed the possibility that the combinations of histone octamer subunits are diverse and chromosomal localization of certain histone octamer species is limited to facilitate DNA-template nuclear events (23). Chromatin reorganization comprises two processes. The first is histone octamer transfer, most evident when daughter DNA strands assume their proper nucleosomal structure immediately after DNA replication (24). In the case of DNA repair, canonical histones in chromatin regions harboring damaged DNA are replaced by specific histone octamer units including specifically modified histones or histone variants (25). Such histone octamer eviction and transfer also occur at gene promoters, where nucleosomal rearrangement is required to facilitate transcriptional activation or inactivation. The second process is histone octamer sliding. Histone octamers slide while chromosomal DNA continuously winds (26). These two processes are likely conducted by means of ATP-dependent chromatin remodeling complexes and
histone chaperones. The chromatin remodeling complexes are classified into four groups, based on the major catalytic subunits, the ATPases (Fig. 2). Each group complex appears to consist of core subunits and cell type-specific regulatory factors (27). For instance, the tissue specificity of the switching defective/sucrose nonfermenting (SWI/SNF) complex in living animals depends on cell type-specific p60 subunits (28). The Mi2-type complex probably induces chromatin inactivation owing to inclusion of histone deacetylases (HDACs), while the SWI/SNF- and imitation switch (ISWI)-type complexes appear to be involved in both chromatin activation and inactivation (14,29;30). At the present time, a number of histone chaperones have been reported to assist in the eviction and assembly of histone octamers into chromatin (20,31;32). However, the exact roles of histone chaperones in chromatin reorganization remain largely unknown.

Histone Modifications and the Histone Code

The N-tails of histones extend outside the DNA-histone octamers and serve as substrates for a variety of histone-modifying enzymes. Post-translational modifications of histone tails include eight chemical modifications, including acetylation, methylation and ubiquitination (Fig. 3). These histone modifications by chemical moieties of small molecular weights are reversible (33). Certain combinations of histone modifications constitute a non-DNA genetic code (the “histone code”) (34). In transcriptionally active euchromatin, histones at gene promoters are methylated at histone H3 lysine 4 (H3K4 Me) and 36 (H3K36 Me) residues in addition to histone H3 hyperacetylation. On the other hand, in inactive heterochromatin, methylations at H3K9 and K27 (H3K9 Me and H3K27 Me) and H3 hypoacetylation are common (35). In addition, histone monoubiquitination probably facilitates the elongation process in transcription (36). Histone modifications appear to “cross-talk” and to be altered in response to intracellular and extracellular conditions.
Fig. 3. Histone modifications involved in chromatin reorganization. Histone N-tails are post-translationally modified, and certain combinations of histone modifications appear to generate a "histone code" defining the chromatin state.

Fig. 4. Histone methylations and their related enzymes. Histone H3 tails are the best established link to gene regulation. H3 methylations are critical determinants for the state of chromatin. At the same lysine residue, several methyltransferases as well as demethylases have been identified. Lysines can be mono-, di- or trimethylated. ASH1: absent small and homeotic disks protein 1 homolog; MLL: myeloid/lymphoid or mixed-lineage leukemia; SET: Suvar3-9, Enhancer-of-zeste, Trithorax; NO66: nucleolar protein 66; JARID1: Jumonji, AT rich interactive domain 1; FBXL: F-box and leucine-rich repeat protein; LSD1: lysine-specific histone demethylase 1; SETDB1: SET domain, bifurcated 1; SUV39h1: suppressor of variegation 3-9 homolog 1; PHF8: plant homeo domain finger protein 8; JMJD: Jumonji domain-containing; EZH2: enhancer of zeste homolog 2; UTX: ubiquitously transcribed X chromosome tetratricopeptide repeat protein; SMYD2: SET and MYND domain-containing 2; SETD2: SET domain containing 2; NSD1: nuclear receptor binding SET domain protein 1.
conditions (37). The molecular basis of such cross-talk and dynamic alterations in histone modifications are poorly understood. However, at the present time, it is thought that the most upstream histone modification to affect downstream patterns is histone methylation (38) (Fig. 4).

In transcriptional events, H3K4, K9, K27 and K36 residue methylations are likely the most significant hallmarks. The regulation process of methylations at these residues appears highly complicated, as up to three methyl moieties can be transferred at each histone lysine residue. (35). Moreover, multiple histone methyltransferases are reportedly active at the same lysine residue. Similarly, multiple demethylases drive demethylations at the same lysine residues (39;40). Though the physiological impact of each of the histone methyltransferases and demethylases remains to be defined in living animals, it is evident from their cell- and tissue-specific expression patterns that each enzyme has a unique role in physiological and pathological processes.

**Histone-Modifying Enzymes That Serve as Transcriptional Co-regulators**

Histone-modifying enzymes are regulators of chromatin organization, and indirectly support transcriptional control by DNA-binding transcriptional factors as transcriptional co-regulators (13) (Fig. 1). Likewise, the overt function of the ATP-dependent chromatin remodelers and histone chaperones is transcriptional co-regulation (14;20). It is not surprising that there are numerous transcriptional co-regulators. The environments of gene promoters appear highly diverse and controlling the proper spatio-temporal expression of a given gene requires specific co-regulators (13). Although the outline of gene regulation at the chromatin level is apparent, numerous questions remain. For instance, which occurs first, DNA-binding of DNA-binding transcription factors or chromatin reorganization? What is the most upstream signal for chromatin reorganization? What is the molecular basis underlying a kind of relay among histone modifications? Histone-modifying enzymes often form multisubunit complexes, but what are the roles of non-catalytic subunits in the complexes? In this respect, bone cells are good subjects to probe such questions, but only a few studies have been reported in the bone field.

**The Canonical and Emerging Epigenome Provide New Insights**

DNA methylation is the best-known and well-established chemical modification conveying epigenetic information. DNA methylation patterns in certain chromosomal areas are transmitted across DNA replication cycles to maintain inactive areas of chromatin (41). Methylation of cytosines at CG sites induces heterochromatinization through recruitment of non-histone proteins like HP-1 and histone H3K9 methyltransferases (42). Since histone modifications to define the state of chromatin and their patterns in certain regions of chromatin are conserved beyond generation, histone modifications are considered to be components of the epigenome, which are reversible, unlike DNA methylation (33). In this respect, histone-modifying enzymes as well as ATP-dependent chromatin remodeling complexes appear to serve as epigenetic regulators. This idea is supported by data showing that the co-regulator functions of the epigenetic regulators are generally shared with multiple classes of DNA-binding transcription factors, and some of them act as global regulators for chromatin reorganization.

**Osteoblastic Differentiation Facilitated by the ATP-Dependent Chromatin Remodeler, the SWI/SNF-Type Complex**

From accumulating evidence of the importance of chromatin remodelers in chromatin reorganization, which associates with gene regulation, it is obvious that differentiation processes of each different bone cell type in adult bone mediate chromatin reorganization conducted by chromatin remodelers. Though a number of DNA-binding transcription factors have been shown to determine bone cell fate, chromatin reorganization at the target gene promoters remains to be studied in terms of
the action of chromatin remodelers. Recently, pioneering work was reported by Flowers et al. (43). Using MC3T3-E1 cells, the role of the catalytic ATPase subunits, Brahma (BRM) and BRM/SWI2-related gene 1 (BRG1), in the SWI/SNF-type complex, was examined by characterizing the osteocalcin promoter during osteoblastic differentiation. In \textit{in vitro} systems, BRM and BRG1 have similar chromatin remodeling activities, activating (loosening) or inactivating (packing) chromatin structure. However, the two complexes exhibited opposing functions during MC3T3-E1 cell maturation when mineralization and alkaline phosphatase activities were monitored. In early stages of differentiation, the BRM-containing complex acted as a repressor, recruiting HDAC1 to the osteocalcin gene promoter in differentiated cells. Though the target transcription factors for these complexes have not yet been identified, it is most likely that the SWI/SNF-type complexes facilitate osteoblastic differentiation through chromatin reorganization (Fig. 5). In this respect, this study demonstrates the molecular function of chromatin remodelers in bone cell differentiation.

\begin{center}
\textbf{Fig. 5.} Chromatin remodeling in osteoblasts. SWI/SNF-type complexes facilitate activation of the osteocalcin gene promoter in osteoblasts. BRM: Brahma; BRG1: BRM/SWI2-related gene 1; RUNX2: runt-related transcription factor 2; ARID: AT-rich interactive domain.
\end{center}

\textbf{HDAC4 in Chondrogenesis of Developing Bones}

As anticipated from the physiological impact of HDAC family members in the differentiation of specific cell types, HDAC4 was identified as the first histone-modifying enzyme to determine bone cell type. In fact, HDAC4-null mice displayed premature ossification caused by ectopic and early onset chondrocyte hypertrophy. Since HDAC4 was shown \textit{in vitro} to co-repress Runx2, a major chondrogenic transcription factor, it appears that HDAC4 is a prime repressor in chondrocyte hypertrophy (44). As chondrogenesis is highly regulated by many transcription factors, it is entirely possible that other HDACs also serve as transcriptional co-repressors to attenuate the function of chondrogenic transcription...
factors until differentiation stages, when such chondrogenic factors start to act. On the other hand, histone acetylases (HATs) are likely to serve as transcriptional co-activators in bone cell proliferation and differentiation, presumably as global co-activators, since the bone-specific function of HATs has yet to be reported.

**Histone Methylation/Demethylation: Do Epigenetic Marks Govern Bone Cell Fate?**

Polycomb group (PcG) proteins were originally discovered in *Drosophila* as epigenetic repressors for Hox genes that are essential for pattern formation of developing embryos. PcG proteins, which are conserved across metazoans, form polycomb repressive complexes (PRCs)1-4 in mammals (45). These complexes are believed to be essential components for developing bone similar to other organs in developing embryos. Recent biochemical analysis of PRCs has found that a histone H3K27 methyltransferase (EZH2: enhancer of zeste homolog 2) as well as HDACs are core subunits of PRCs, indicating that their repressive function mediates chromatin modifications that are directing chromatin inactivation (46). Consistent with the global repressive roles of PRCs in chromatin organization in organogenesis of developing embryos, the core component Bmi-1 reportedly affects osteoblastic differentiation (47).

Fig. 6. Histone demethylase is a negative regulator for Osterix (Osx)-mediated gene regulation in osteoblastogenesis. A histone H3K4 and K36 demethylase, nucleolar protein 66 (NO66), serves as a transcriptional co-repressor for Osx, a critical inducer of osteoblastogenesis. Through demethylation of active histone H3K4 and H3K36 methyl groups, surrounding chromatin areas are likely inactivated as a transcriptional control.

It is currently believed that the pattern of histone methylation marks plays a central role in gene regulation during bone cell differentiation. Indeed, genetic manipulation has shown that a core subunit (WDR5: WD-repeat domain 5) of the histone-activating H3K4 methyltransferase complex family (MLL1-4) contributes significantly to osteoblastogenesis (48;49). However, as there are a number of histone methyltransferases and demethylases, the roles of each enzyme in bone cell differentiation and proliferation are not clear. Based upon the HDAC4 study, histone
methylation-related enzymes seem to co-regulate the function of osteo-transcription factors. This concept is currently gaining support. A histone H3K9 methyltransferase, SET domain bifurcated 1 (SETDB1), was found to co-repress PPARγ (a prime adipogenic factor), while co-activating Runx2, resulting in a mesenchymal stem cell switch from adipogenesis to osteoblastogenesis. Notably, SETDB1 enzymatic activation was needed to form a complex upon Wnt signal activation, representing a system where histone-modifying enzyme activity is under control at a level of complex formation, in response to an extracellular signal (16). More recently, the histone H3K4 and K36 demethylase nucleolar protein 66 (NO66) was identified biochemically as a directly-associating co-repressor for Osterix, an essential transcription factor in osteoblastogenesis (50). As NO66 co-repressed Osterix through demethylation of active histone methylation marks, NO66 is proposed to be a negative regulator in the Osterix-mediated gene cascade (Fig. 6).

**Perspective**

Dynamic alterations in histone modification patterns during bone cell differentiation are now evident. The factors involved in chromatin reorganization are most likely essential for bone development. Such factors have often been shown to form multisubunit complexes that are serving as chromatin remodelers and histone-modifying enzymes (16). However, how many complexes exist, and the precise roles of the subunits, remain unclear. Since complex subunits are expressed in a cell type-specific manner, it will be intriguing to define those subunits that are specific for bone cells and their precursors. Identification of osteo-epigenetic factors will uncover new aspects of bone biology, and provide new directions for bone biology research in regenerative medicine and drug discovery.

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**References**


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