PROSPECTS
Journal of Cellular Biochemistry 114:975–984 (2013)

Journal of Cellular Biochemistry

Genetic and Molecular Control of Osterix in Skeletal Formation

Krishna M. Sinha[1*] and Xin Zhou[2]

[1] *Department of Endocrine Neoplasia and Hormonal Disorders, UT MD Anderson Cancer Center, Houston, Texas 77030*
[2] *Department of Genetics, UT MD Anderson Cancer Center, Houston, Texas*

ABSTRACT

Osteoblast differentiation is a multi-step process where mesenchymal cells differentiate into osteoblast lineage cells including osteocytes. Osterix (Osx) is an osteoblast-specific transcription factor which activates a repertoire of genes during differentiation of preosteoblasts into mature osteoblasts and osteocytes. The essential role of Osx in the genetic program of bone formation and in bone homeostasis is well established. *Osx* mutant embryos do not form bone and fail to express osteoblast-specific marker genes. Inactivation of *Osx* in mice after birth causes multiple skeletal phenotypes including lack of new bone formation, absence of resorption of mineralized cartilage, and defects in osteocyte maturation and function. Since Osx is a major effector in skeletal formation, studies on Osx gained momentum over the last 5–7 years and implicated its important function in tooth formation as well as in healing of bone fractures. This review outlines mouse genetic studies that establish the essential role of Osx in bone and tooth formation as well as in healing of bone fractures. We also discuss the recent advances in regulation of *Osx* expression, which is under control of a transcriptional network, signaling pathways, and epigenetic regulation. Finally, we summarize important findings on the positive and negative regulation of Osx's transcriptional activity through protein–protein interactions in expression of its target genes during osteoblast differentiation. In particular, the identification of the histone demethylase NO66 as an Osx-interacting protein, which negatively regulates Osx activity opens further avenues in studying epigenetic control of Osx target genes during differentiation and maturation of osteoblasts. J. Cell. Biochem. 114: 975–984, 2013.

KEY WORDS: OSTERIX; OSX; NO66; GENE EXPRESSION; OSTEOBLASTS; OSTEOCYTES

Bone formation occurs through two developmental processes—intramembranous and endochondral ossification. Intramembraneous ossification which mainly forms craniofacial bone is mediated by the differentiation of the condensed mesenchyme into osteoblasts. In endochondral ossification, mesenchymal cells in the condensation differentiate into chondrocytes, forming a cartilage template that is later replaced by bone [Fang and Hall, 1997]. In skeletogenesis, Sox9, Runx2, and Osterix are three essential transcription factors that have important roles in the cell-fate decision process by which mesenchymal cells become chondrocytes and osteoblasts, through activation of cell type-specific genes. Sox9 is the earliest expressed transcription factor in prechondrogenic mesenchymes, which differentiate into chondrocytes. Runt-domain-containing Runx2, another early differentiation factor, is expressed in the condensed mesenchymes. *Runx2*-expressing cells segregate from osteochondroprogenitors to form precursor osteoblasts. Expression of *Osx* in *Runx2*-expressing precursors induces these cells to differentiate into mature and functional osteoblasts, and finally into osteocytes during bone formation [Karsenty, 2001; Nakashima et al., 2002; Yang and Karsenty, 2002; Nakashima and de Crombrugghe, 2003; Nishio et al., 2006; Zou et al., 2006; Zhou et al., 2010]. Several other transcription regulators including Wnt/β-catenin, homeodomain protein Dlx, Msx2, ATF4, and bone morphogenetic proteins have been known to play important roles in controlling gene expression during differentiation and maturation of osteoblasts [Yang et al., 2004; Rodda and McMahon, 2006; Karsenty, 2008; Hassan et al., 2009]. In this review, we will primarily focus on the role of Osx in control of genetic and molecular programs of osteoblast differentiation.

CONTROL OF Osx IN THE GENETIC PROGRAM OF BONE FORMATION

Over a decade ago, the lab of de Crombrugghe identified Osterix (Osx/sp7) cDNA using a differential hybridization approach from

Grant sponsor: ; Grant numbers: AR049072, AR061590.
*Correspondence to: Krishna M. Sinha, Department of Endocrine Neoplasia and Hormonal Disorders, UT MD Anderson Cancer Center, Houston, TX 77030 E-mail: ksinha@mdanderson.org
Manuscript Received: 29 June 2012; Manuscript Accepted: 23 October 2012
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 5 December 2012
DOI 10.1002/jcb.24439 • © 2012 Wiley Periodicals, Inc.

BMP2-treated and untreated C2C12 cells [Nakashima et al., 2002]. BMP2 treatment induces differentiation of C2C12 cells into osteoblasts by stimulating expression of osteoblast genes. Molecular characterization of this cDNA led to the identification of Osx, which contains a proline-rich activation domain at the N terminus spanning aa 1-289 and three C_2H_2-type zinc finger DNA-binding domains at the C terminus. In DNA transfection studies, Osx containing aa 1-172 is sufficient for transcriptional activation. Osterix is sometimes referred to as Sp7 because of the striking homology with zinc finger DNA-binding domains of Sp1, Sp3, and Sp4 transcription factors. During mouse embryonic development, *Osx* expression begins around E13.5 in calvaria and perichondrium of long bones and is robustly expressed at E15.5 in all osseus elements. *Osx* is also expressed at a lower level in pre- and hypertrophic chondrocytes. It is clear that Runx2 is required to prime the prechondrogenic mesenchyme segregate into the precursor osteoblast lineage, whereas Osx is subsequently required to accomplish the osteoblast differentiation pathway (see Fig. 1).

Osx-null cells are arrested in their differentiation: *Osx*-null embryos either do not express osteoblast markers or express them at very low levels, despite the normal expression of *Runx2*. Expression of *Col1a1*, which is expressed at higher levels in mesenchyme of wild-type mice, is significantly reduced in *Osx* mutants. Other osteoblast markers including *Osteonectin*, *Osteopontin* and *Bone-sialo protein* are undetectable in endochondral and membranous skeletal elements in *Osx* mutants. *Osteocalcin*, the very late and specific osteoblast marker normally expressed at E18.5, is also absent from prefigured skeletal zones in *Osx*-null embryos. Expression of *Runx2* is also normal in the condensed mesenchyme of membranous and endochondral skeletal zones in *Osx*-null

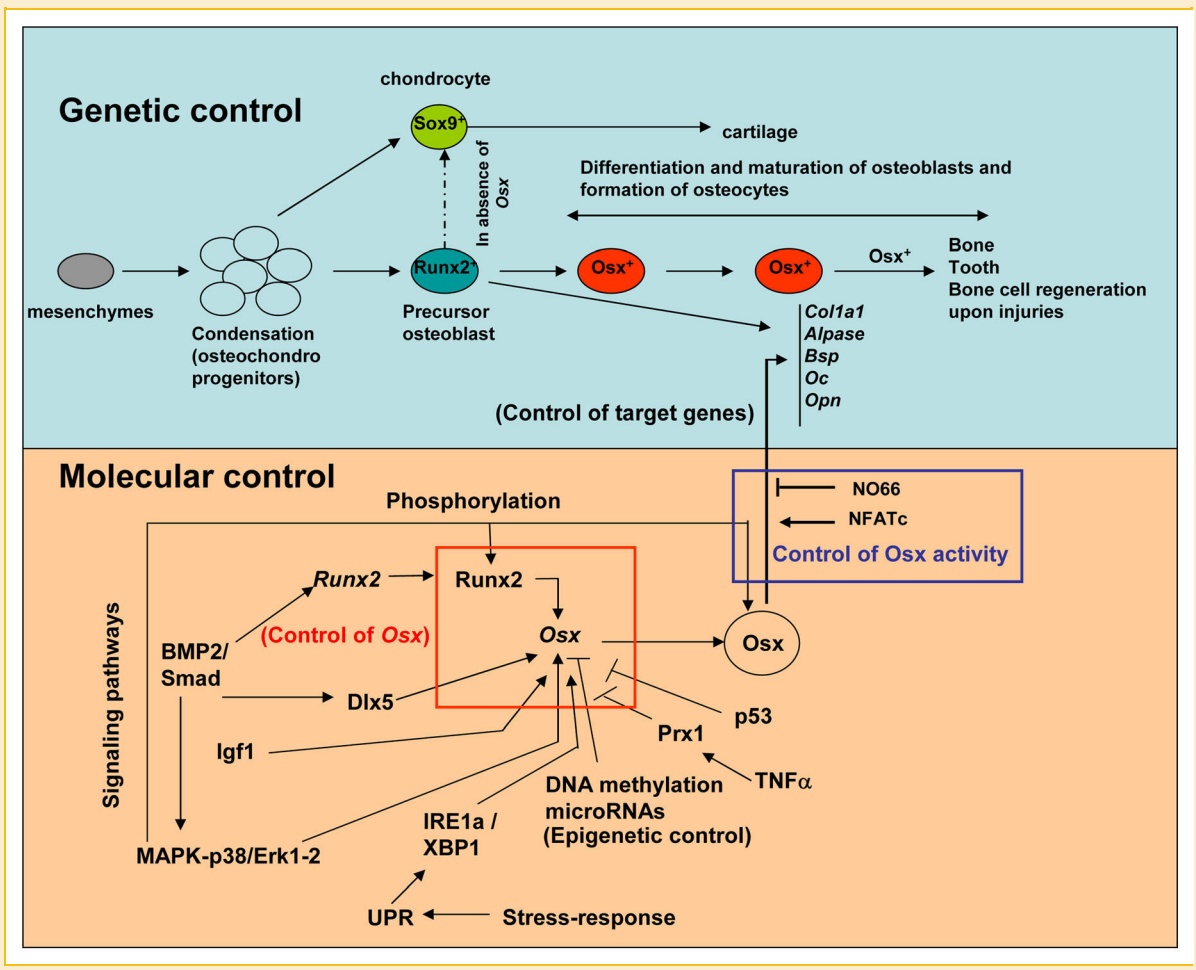

Fig. 1. Osteoblast differentiation is a multi-step process. Multipotent mesenchymal precursors in condensed mesenchymes differentiate into *Sox9*-expressing osteochondroprogenitors, which segregate into chondrocytes that express high levels of Sox9, and preosteoblasts that express high levels of *Runx2* and low level of *Col1a1* and *Akp*. Osx expression is needed to accomplish the differentiation of preosteoblasts to mature and functional osteoblasts that express high levels of osteoblast markers including *Col1a1*, *Akp*, *Bsp*, and *Oc*. Osx controls the genetic program of osteoblast differentiation (shown in light blue area). Functional osteoblasts produce ECM molecules which form a calcified bone matrix. *Osx*-null cells are arrested in their differentiation and acquire chondrocytes phenotype by expressing chondrocyte markers. The molecular control of Osx is depicted in light yellow area. Expression of *Osx* mRNA and protein is positively regulated by signaling pathways (BMP2, MAPK, and Igf1), Runx2, Dlx5, ER-stress pathway (IRE1a/XBP1), and negatively regulated by DNA methylation and microRNAs, p53, and TNF. The transcriptional activity of Osx is documented by NFATc as positive regulator and by NO66 histone demethylase as negative regulator.

embryos and long bones, but *Osx* is evidently not expressed in skeletal parts of *Runx2*-null embryos, indicating that Runx2 is an upstream regulator of *Osx*. These studies strongly indicate that Osx triggers *Runx2*-precursors to develop into osteoblasts by activating expression of osteoblasts genes [Nakashima et al., 2002].

Is Osx a negative regulator of chondrocytes? *Osx*-null embryos, unlike *Osx*-expressing embryos, do not form bone but apparently chondrocyte differentiation and cartilage formation are not affected in null embryos. *Runx2*-positive *Osx*-null cells express chondrocyte-specific genes such as *Sox9* and *Col2a1* and acquire a chondrocyte-type cell fate [Nakashima et al., 2002]. These observations lead to speculation that Osx inhibits chondrocyte differentiation in *Runx2*-expressing precursor osteoblasts. *Runx2* is expressed early on in prechondrogenic mesenchyme cells, which express high levels of *Sox9*. Later on, *Osx* expression is stimulated, *Runx2*-positive cells exit as precursor osteoblasts and *Sox9* expression is down-regulated, and thereby suggesting that expression of *Sox9* and *Osx* is mutually exclusive. Those *Runx2*/*Osx*-expressing cells strictly differentiate into mature and functional osteoblasts in which *Sox9* is not expressed. The *Runx2*-expressing precursors, prior to *Osx* expression, remain in the chondrogenic lineage and do not progress into the preosteoblast stage. The spatial and temporal expression of *Osx*, studied by Kaback et al. [2008], also indicates that the increase in *Osx* expression specifically coincides with the onset and progression of osteoblast differentiation but occurs after the maturation of chondrocytes [Kaback et al., 2008]. In addition, the levels of *Osx* mRNA remain high after cartilage is formed and remodeled into bone. These studies suggest that Osx plays dual roles in inhibition of chondrocyte formation and in promoting osteoblast differentiation.

Essential role of Osterix in bone homeostasis in adults: Osx also plays an essential role in formation of adult bones and in expression of osteoblast genes in the adult. Zhou et al. demonstrated that inactivation of *Osx* in adult mice, using LoxP/inducible Cre activity, causes multiple skeletal phenotypes including lack of new bone formation, abnormal cartilage accumulation beneath the growth plate, and defects in osteocyte maturation and function. In addition to down-regulation of osteoblast genes in *Osx*-ablated adult mice, expression of osteocyte-specific marker genes including *Sost*, *Dkk1*, *Dmp1*, and *Phe* is decreased. These studies reveal that a new role of Osx in resorption of cartilage matrix eventually replaced by bone matrix. Note that this strategy was used to delete *Osx* in all cell types using *pCMV Cre-ERT2* [Zhou et al., 2010]. Role of Osx in degradation of cartilage matrix was further implicated by another study indicating that Osx plays important role in calcification of cartilaginous matrix by directly targeting activation MMP-13 in the hypertrophic chondrocytes of the growth plate during endochondral bone formation. Conditional and global ablation of Osx caused the loss of MMP-13 expression and arrest of endochondral ossification at hypertrophy stage [Nishimura et al., 2012]. When *Osx* is inactivated specifically in osteoblasts using 2.3 kb *Col1a1-Cre* during embryonic development, no apparent skeletal defects were observed in newborn mice. However, in growing adult stage mice 8 weeks old or older, *Osx* mutant mice displayed reduced bone volume in trabecular and lumbar vertebra and decreases in thickness and number of trabecular bone, thus exhibiting human skeletal defects similar to osteopenia [Baek et al., 2009]. Further, inactivation of *Osx* in osteoblasts of adult mice with inducible Cre recombinase driven by *Col1a1-Cre ERT2* also causes such skeletal phenotypes as reduced expression of osteoblast genes, reduced osteoblast differentiation and function, and mild reduction in bone loss [Baek et al., 2010]. Taken together, these studies depending upon time of *Osx* inactivation clearly indicate that Osx continues to play a role as an anabolic regulator that supports the homeostasis of bone in adults.

Although ablation of *Osx* in mice causes inhibition of bone formation, transgenic mice that over-express *Osx* by 2.3 kb *Col1a1* promoter show osteopenia, characterized by woven bone structure in the cortical bone, as well as decreased expression of osteoblast genes, especially *Oc*. In *Osx*-expressing transgenic mice, osteoblast differentiation is inhibited and immature osteoblasts are accumulated. These results indicate that proper dosages of Osx are required to promote osteoblast differentiation and bone formation in adults [Yoshida et al., 2012].

MUTATIONS IN *Osx* ARE ASSOCIATED WITH HUMAN SKELETAL DISEASES

Genetic analyses using GWAS, SNP, and linkage mapping are used to identify genes that cause human diseases. Skeletal disorders such as osteoporosis and osteogenesis imperfecta (OI) are associated with mutations in causative genes, including type I collagen gene, which causes low bone mass, and bone mineral density (BMD) and makes the animal susceptible to multiple bone fractures. Lapunzina et al. identified a homozygous single nucleotide deletion (1052delA) mutation in *OSX* gene of an Egyptian child who had been clinically diagnosed with OI. This patient had repeated fractures, mild bone deformities, delayed tooth eruption, etc. *OSX* mutation occurred at the third zinc finger of the DNA-binding domain, causing a frameshift mutation that produced a truncated OSX protein. The reduced activity of OSX may have altered expression of the OSX target bone-specific genes including *type1 collagen* in this patient [Lapunzina et al., 2010]. Genome-wide association studies further revealed a number of genetic variants associated with BMD and osteoporosis. Among several GWAS-derived loci, *OSX* is the only locus containing genetic variants over-represented in female childhood obesity for increased BMD. Further SNPs in the *OSX* region indicate a strong correlation between genetic variants at the *OSX* locus with adult lumbar spine and BMD [Timpson et al., 2009; Zhao et al., 2011]. These genetic, clinical, and epidemiological studies indicate the significance of Osx for overall bone health in adults. Further studies are required to identify mutations in the *OSX* gene from patients with bone disorders to solidify the correlation between Osx mutations and bone diseases.

Osx IS NEEDED FOR ODONTOBLAST DIFFERENTIATION AND TOOTH DEVELOPMENT

The process of tooth formation is quite similar to that of bone formation. Dental mesenchyme cells differentiate into odontoblasts, which produce and secrete collagenous and non-collagenous matrix

protein during dentin formation. Dentin sialo phospho protein is a major component of non-collagenous dentin matrix protein and essential for dentin formation. Mouse genetic and biochemical studies demonstrated that both Runx2 and Osx have important roles in tooth formation and function independently in activation of specific genes required for odontoblast differentiation [Zhao et al., 2007; Chen et al., 2009; Hirata et al., 2009]. Chen et al. showed that expression of *Dsp* is positively correlated with that of *Osx*, but not with *Runx2*. *Runx2* and *Osx* expression overlap in alveolar bone osteoblasts, odontoblast, amyloblasts, and dental pulp cells in E16 mouse embryos, when *Dsp* is weakly expressed in preodontoblast. By the later stage of differentiating and mature odontoblasts at E18, expression of *Runx2* dramatically decreases, whereas *Osx* expression remains intense in odontoblast and dental pulp cells in which *Dsp* expression is strongly expressed. It appears that Osx is needed for differentiation and maturation of preodontoblast to odontoblast through activation of specific late marker genes. This situation of Osx function is reminiscent of osteoblast differentiation where Osx is needed for differentiation of preosteoblast to osteoblasts. Overlapping expression of *Osx* and *Dsp* is also observed at the postnatal stage of tooth development. Forced expression of *Osx* in mouse odontoblast-like cells stimulates expression of *Dsp* mRNA, indicating that the *Dsp* gene could be a direct target of Osx activation [Chen et al., 2009]. Future studies such as RNASeq in combination with ChIP sequencing are required to validate indications that Osx directly activates expression of odontoblast genes during tooth formation.

In the last 5 years, Osx has generated much attention in bone as well as dental research. Recently, Cao et al. used mouse models to elucidate for the first time Osx's role in the formation of cellular cementum—a mineralized tissue that supports tooth development. Transgenic mice that overexpress Osx driven by the 3.6 kb *Col1a1* display an increase in cementum formation, whereas tissue and stage-specific deletion of *Osx* in mice with 2.3 kb *Col1a1-Cre* in embryos or with *CMV-Cre ERT2* in adults showed sharp declines in cementum formation and expression of *Dmp1*. These data further support the important function of Osx in tooth formation [Cao et al., 2012].

POTENTIAL USE OF OSTERIX IN BONE FRACTURE AND REPAIR

Given that Osterix is a classic osteoblast marker and essential for bone formation, exploitation of Osx as an anabolic molecule is an appealing avenue in the repair of bone fractures and injuries. Bone repair is a complex process that includes spatial and temporal expression of multiple molecules and interactions of these molecules in formation of new bone during fracture healing. A review on gene therapy approaches in bone regeneration with recent works on molecules that are required for bone healing is summarized [Franceschi, 2005]. Studies involving controlled bone injuries followed by healing in animals have demonstrated that *Osx* is expressed intensely during regeneration of bone cells at fracture sites. Implanting of *Osx*-expressing BMSc (bone marrow stroma cells) at the surgically incisor of rat calvaria accelerates bone healing at the injured calvaria much more than implanted control BMS cells by forming new bone at the injured calvaria as well as by inducing expression of bone-specific genes [Tu et al., 2007]. Further, *Osx*-expressing BMSc cells implanted into nude mice also induced bone formation through acceleration in osseo integration of implants [Tu et al., 2006; Xu et al., 2009]. Kaback et al. showed that in mice with controlled bone fracture, the fracture callus and cartilage began to form at Day 7 after fracture and continued at Day 10 with an increase in *Sox9* mRNA. *Osx* was expressed in the osteoblast of the woven bone of the fracture callus at day 10, but was excluded from cortical bone, hypertrophic chondrocytes, and blood vessels. At Day 14 post-fracture, *Osx* was mostly expressed in osteoblasts surrounding the healed sites whereas expression of *Sox9* decreased and cartilage of the callus remodeled into bone [Kaback et al., 2008]. In the same study, treatment of PTHrP or BMP2 to osteochondro progenitors which were derived from mouse limb buds that expressed high levels of *Sox9* induced expression of *Osx* but PTHrP or BMP2 decreased that of the chondrocyte markers *Sox 9* and *Col2a1*. Over-expression of *Osx* in these progenitors decreased *Sox9* level, suggesting that Osx might inhibit chondrocyte differentiation while promoting osteoblast differentiation [Kaback et al., 2008].

The mechanism of migration of precursor osteoblasts at the primary ossification site during normal bone formation or fracture healing was further demonstrated by lineage tracing approach using two mouse strains, harboring Osx-CreERt and Col1a1-ERt transgenes, and crossing these mice with Rosa26R-LacZ reporter [Clarkin and Olsen, 2010; Maes et al., 2010]. Injection of 4-OH tamoxifen to transgenic mice at specific time during development activates expression of Cre recombinase which subsequently induces *LacZ* expression [Maes et al., 2010]. In this study, authors reported that two populations of osteoblast-lineage cells exist at the periochondrium; the precursor osteoblasts which are *Osx*-positive, and the differentiated osteoblasts which are *Col1a1*-positive. These experiments showed that Osx/LacZ expressing precursor osteoblasts were sparsely present in the cortex but were abundantly present inside the trabecular bone whereas Col1a1/LacZ cells were enriched at the cortex and engaged into cortical bone formation and were hardly present in the trabecular bone. This study proposes a mechanism by which precursor osteoblasts (Osx-/LacZ positive cells) enter at the site of primary ossification center along with blood vessels because *Osx*-expressing precursors are intimately associated with endothelial cells of blood vessels. In addition, bone fracture studies performed in Osx-Cre:GFP mice that allowed tracing of GFP positive cells further revealed that before fracture these Osx-GFP positive cells were present in the thin periosteal layer of the cortical bone, but after fracture these cells along with blood vessels were massively localized to the woven bone callus at the healing regions. These studies indicate the potential application of Osx as bone regenerative medicine in healing of bone fractures.

Osx EXPRESSION IS DEPENDENT ON OR INDEPENDENT OF Runx2 ACTIVITY

In *Osx*-null embryos *Runx2* is expressed whereas in *Runx2*-null embryos, *Osx* is not expressed, and therefore, Osx can be placed

downstream of Runx2, where it activates *Osx* expression (see Fig. 1). Sequence analysis of the *Osx* promoter reveals that several putative Sox9, VDRE, Runx2, Dlx5 and Sp1 response elements are present within upstream regulatory sequence of the *Osx* gene [Nishio et al., 2006]. In a reporter assay, Runx2 was able to activate the *Osx* promoter through direct interactions with Runx2-binding element present within 1 kb of the *Osx* promoter. Other studies also indicated that *Osx* expression is not exclusively dependent on Runx2 activity. In *Runx2*-null calvarial cells in which *Osx* is normally not expressed, BMP2 treatment induced *Osx* expression through upregulation of Dlx5, which interacted with *Osx* promoter containing Dlx5 binding elements [Lee et al., 2003]. BMP signaling did not directly induce expression of *Osx* but through phosphorylation of Dlx5 enabled the interactions of Dlx5 with the *Osx* promoter. These studies indicate that *Osx* expression is mediated through alternate pathways independently of Runx2. It would be reasonable to conclude that, although Runx2 alone may not be sufficient for *Osx* expression, it functions in tandem with other signaling pathways to mediate Runx2-independent Osx expression during osteoblast formation. Interestingly, Osx regulates its own expression through a feedback mechanism by interacting with its own promoter [Yoshida et al., 2012].

Osx expression under control of signaling pathways: several studies elucidated the role of bone morphogenetic protein 2 (BMP2) and insulin-like growth factor (Igf1) signaling pathways in activation of osteoblast genes during osteoblast differentiation. These growth factor-ligand complexes elicit a cascade of signal transduction pathways followed by phosphorylation of downstream substrates to control gene transcription [Jadlowiec et al., 2004; Huang et al., 2007]. BMP2 is known to induce *Osx* expression in a Runx2-dependent manner. BMP2/Smad pathway targets activation of *Runx2*, which in turn activates *Osx* expression. Igf1 also up-regulates *Osx* expression without altering *Runx2* expression. Although Igf1 response in *Osx* expression is not as strong as BMP2, a combination of BMP2/Igf1 synergistically increases *Osx* expression in human mesenchymal stem cells (hMSC) compared with Igf1 alone. This suggests that *Osx* expression requires other signaling pathways beyond BMP2/Smad/Runx2. The synergistic activation of *Osx* expression is most likely due to common convergent mitogen-activated protein kinase (MAPK) and protein kinase D (PKD) pathways, activated by the BMP2/Igf1 signaling [Celil and Campbell, 2005; Celil et al., 2005]. MAPK has been reported to participate in induction of Alp activity and Ca^{++} deposition during bone formation. There are four members of the MAPK super family—extracellular-signal-regulated kinase 1/2 (Erk1/2), c-Jun amino-terminal kinase (JNK), p38, and Erk5. BMP2 treatment of hMSC activates MAPK components p38 and Erk1/2 through phosphorylation of p38 and Erk1/2, which mediate BMP2-induced *Osx* expression. Inhibition of p38 and Erk1/2 signaling using specific inhibitors down-regulates BMP2-mediated *Osx* expression. Further over-expression of dominant-negative form of p38 or Erk in hMSC completely abolished *Osx* expression under osteogenic media, thereby strongly indicating that *Osx* expression involves the MAPK pathway rather than BMP2/Smad/Runx2 signaling, because MAP/Erk does not inhibit *Runx2* expression. However, activated MAP/Erk does phosphorylate Runx2 to increase the stability, and the transcriptional activity of Runx2 in expression of *Osx*. A recent report further suggests a novel mechanism of *Osx* expression during osteoblast differentiation under osteogenic media by ascorbate-dependent prolyl hydroxylase domain protein (PHD) and proteasome pathway, which targets degradation of yet-to-be-defined transcriptional repressors of the *Osx* gene [Xing et al., 2011]. Ascorbate effects in *Osx* expression are independent of BMP2, Igf1, ECM-mediated integrin signaling, and MAPK pathways, because blockade of either pathway does not affect *Osx* expression. However, inhibition of PHD by the specific inhibitors dimethyloxallyl glycine and ethyl 3,4-dihydroxybenzoate strongly affects ascorbate-induced *Osx* expression and OB differentiation.

Function of tumor-suppressor p53 has also been documented in osteoblast differentiation and bone formation by regulating *Runx2*/*Osx*, supported by both gain of function and loss of functions in mouse models [Liu and Li, 2010]. Wang et al. [2006] showed that tumor-suppressor p53 is able to inhibit *Osx* expression in DNA transfection assays as well as in vivo using mouse genetic approaches. Inhibition by p53 of *Osx* expression does not occur through direct binding on the *Osx* promoter, because there is no p53 binding sequence present in the *Osx* gene. It was suggested that like other target genes, p53 represses *Osx* expression by preventing recruitment of p300 and other activators to the target chromatins. In *p53*-null mice, expression of *Osx*, but not *Runx2*, is up-regulated and so are the other Osx target genes. As a result, the loss of p53 induces accelerated osteoblast differentiation and high bone mass phenotype in *p53* mutant mice [Wang et al., 2006].

ER-stress responds to *Osx* induction: mature osteoblasts produce high levels of extracellular matrix (ECM) proteins during skeletal development, often resulting in accumulation of misfolded proteins that cause endoplasmic reticulum stress (ER stress) [Murakami et al., 2009; Boot-Handford and Briggs, 2010; Park et al., 2012]. To circumvent the ER stress so that ECM proteins may be secreted by osteoblasts, the unfolded protein response pathway is activated by ER stress transducers including IRE1α (inositol-requiring endonuclease 1α), which is involved in processing of transcription factor *XBP1* mRNA [Tohmonda et al., 2011]. XBP1 then binds to UPR elements and activates many genes to alleviate ER stress. The *Osx* promoter also contains the recognition sequence for XBP1 and UPR. Tohmonda et al. [2011] showed that upon treatment of thapsigargin, an ER stress inducer, the *Osx* gene is activated by XBP1 through interaction with *Osx* promoter during BMP-2-induced osteoblast differentiation of mouse embryonic fibroblasts. Overexpression of *XBP1* stimulates *Osx* expression but not Runx2, indicating a functional role of the IRE1a-XBP1 pathway in *Osx* expression during differentiation of preosteoblasts to mature osteoblasts.

Negative regulation of *Osx* expression: epidermal-growth factor receptor (EGFR) and tumor-necrosis factor (TNF) oppose osteoblast differentiation by negatively regulating expression of osteoblast genes. EGFR inhibits *Runx2* expression and consequently *Osx* expression through stimulation of HDAC levels in cells [Zhu et al., 2011]. Several HDACs including HDAC 3-7 are known to inhibit osteoblast differentiation by inhibiting Runx2 activity [Kang et al., 2005; Jensen et al., 2007; Jensen et al., 2008; Bradley et al., 2011]. TNFα promotes bone loss and delayed osteoblast differentiation through upregulation of Prx1 (paired-related homeodomain). Prx1 interacts with the *Osx* promoter and represses *Osx* expression [Lu

et al., 2011]. Characterization of *Osx* gene revealed that two transcription start sites, each driven by independent promoters, give rise to two isoforms of *Osx*; *Osx1* being the more abundantly expressed form compared to alternatively spliced *Osx2*. TNF-response elements are present in both promoters, and the binding of TNF at the *Osx* promoter inhibits *Osx* expression in preosteoblasts MC3T3 cells.

Epigenetic control of *Osx* expression by DNA methylation and miRNAs: DNA methylation is a repressive epigenetic mark that is associated with silencing of the key genes required for cellular differentiation [Minard et al., 2009; Ong and Corces, 2012]. Demethylation of those key genes is essential for activation of gene expression that allows cell-type differentiation during development. A study using cell line-based experiments reported that the promoters of the osteoblast genes including *Dlx5*, *Osx*, *Oc*, and *Alp* are hypomethylated in osteoblast cells that express these genes and are hypermethylated in non-osteogenic cells, which do not express these genes [OLee et al., 2006]. Treatment with azacytidine (5Aza), an inhibitor of DNA methylation, activates expression of silenced osteoblast genes including *Osx* and *Dlx5* in C2C12 mesenchymal cells. This suggests that DNA methylation restricts expression of those genes to prevent the differentiation of multipotent precursors into specific cell lineages [Hupkes et al., 2011]. Hupkes et al. [2011] further reported that the majority of 5Aza-treated C2C12 cells differentiate into myoblasts and to a lesser extent into osteoblasts because 5Aza treatment stimulated expression of the key transcription factors *MyoD* and *Osx* through DNA demethylation. It is likely that hypermethylation of the *Osx* promoter plays a crucial role in imposing a barrier in expression of this gene in pluripotent mesenchymes. Hence, demethylation of the *Osx* promoter might begin to occur in precursor osteoblasts, which results in restriction of its expression to osteoblasts. Along this line, a recent report indicates that an osteocyte-specific marker *Sost* gene is hypermethylated in osteoblasts that do not express *Sost* gene. In osteocytes, the *Sost* promoter is hypomethylated and is expressed [Delgado-Calle et al., 2012]. Note that Osx is needed for the maturation and function of osteocytes by activating osteocyte-specific *Sost*, *Dmp1*, and *Phex* genes [Zhou et al., 2010]. We should speculate that DNA methylation of *Osx* and its target genes is lost at specific developmental stages to ensure transcription of these genes in osteoblasts and osteocytes during bone formation.

MicroRNAs are small noncoding RNAs that negatively regulate gene expression at post-transcriptional and post-translational level by directly targeting mRNA of the key genes, and then subsequently control cellular activities including proliferation and differentiation. A comprehensive review on the role of several miRNAs in skeletal development recently appeared in literature [Lian et al., 2012]. Among those miRNAs, miR-93, 125, 135, 138, 637 have been reported to function in regulation of Osx and to inhibit osteoblast formation [Schaap-Oziemlak et al., 2010; Eskildsen et al., 2011; Goettsch et al., 2011; Yang et al., 2012]. Yang et al. showed that lentivrus-mediated overexpression of miR-93 suppressed osteoblast differentiation and mineral deposition through inhibition of Osx protein, but not Osx mRNA, by directly binding to coding region of *Osx* gene. Interestingly, this study also revealed that expression of miR-93 gene is under the control of Osx activity because Osx interacted with the promoter of miR-93 gene and overexpression of Osx suppressed miR-93 expression. These data indicate that that miR-93 regulates differentiation program of osteoblast through Osx/Sp7 feedback mechanism [Yang et al., 2012]. Another miR-637 is overexpressed in adipocytes and suppressed Osx expression in these cells by directly targeting the 3UTR of the *Osx* gene. Thus, miR-637 promotes transdifferentiation of mesenchymes cells into adipocytes by inhibiting Osx expression and then subsequent osteoblast differentiation [Zhang et al., 2011]. Others miRNA-125b, 135b, and 138 have also been reported to negatively regulate Osx expression directly or indirectly in non-osteoblasts such as vascular smooth muscle cells, unrestricted somatic cells, or human stromal mesenchymal cells, all of which have potential to differentiate into osteoblasts in which these miRNAs are down-regulated.

CONTROL OF Osx TRANSCRIPTIONAL ACTIVITY

Given that Osx was discovered over a decade ago and Osx is a recognized key regulator of bone formation, we are dismayed that we have such limited knowledge of the molecular mechanisms of Osx activity in activation of osteoblast genes. In this section, we will update our knowledge from recent studies of the regulation of Osx activity.

NFTAc, TFII, p300, and Brg1 promote Osx activity: nuclear factor of activated T cells (NFATc), an essential transcription factor for osteoclast differentiation, also plays important role in osteoblast differentiation through positive regulation of Osx activity. NFATc induces Osx-dependent activity of its target promoter 2.3 kb *Col1a1* in reporter assays. NFATc interacts with the activation domain of Osx and this interaction is needed for recruitment of NFATC at the Osx-target *Col1a1* promoter. In Osx knocked-down cells (by siRNA), occupancy of NFATc at the *Col1a1* promoter is sharply decreased. Further, there is no activation of the 2.3 kb *Col1a1*-reporter in *NFATc−/−* primary osteoblasts, suggesting that NFATc acts positively in osteoblast differentiation by stimulating Osx activity [Koga et al., 2005]. Other study shows that the Osx fragment with aa 141–210 is sufficient for activation and interacts with TFII and that the C terminus of Osx (280–428aa) interacts with Brg1, a catalytic component of SWI/SNF complexes of ATP-dependent chromatin remodeling activity [Hatta et al., 2006].

NO66 histone demethylase inhibits Osx activity: NO66 is a jumonji C containing protein identified as Osx-interacting polypeptides using a proteomic approach and mass spectrometry. NO66 exhibits demethylase activity in vitro with dual specificity for lysine 4 and 36 of histone H3 [Sinha et al., 2010]. NO66 is a part of the preribosome complex, localized mainly in nucleoli but also in nucleoplasm, ubiquitously expressed in all tissues, and evolutionarily conserved among vertebrates [Eilbracht et al., 2004]. Histone demethylases control the levels of histone methylation in chromatin and regulate the transcriptional state of the gene in various cellular activities including proliferation, differentiation, senescence, etc. [Martin and Zhang, 2005; Bernstein et al., 2006; Klose et al., 2006; Tsukada et al., 2006; Benevolenskaya, 2007; Cloos et al., 2008]. Along these lines, the interactions of NO66 demethylase with Osx should be considered physiologically significant in regulating osteoblast differentiation through modulation of Osx activity [Sinha et al., 2010].

Interactions between Osx and NO66 occur through the activation domain of Osx and JmjC domain, including the C terminus of NO66, in vitro and in vivo. In DNA transfection assays, NO66 inhibits the Osx-dependent activation of the Osx-target *Bsp* and *Oc* promoters. Knock-down of NO66 by shRNA in preosteoblast MC3T3 cells accelerates osteoblast differentiation in these cells by stimulating both *Alp* activity and ECM deposition. Expression of *Col1a1*, *Bsp*, and *Oc* genes is significantly up-regulated in NO66-depleted MC3T3 cells. NO66 inhibits expression of Osx target osteoblast genes likely through interaction with Osx and through regulating histone methylation levels in the chromatin of these genes. Chromatin immunoprecipitation (ChIP) assays indicate that there is an inverse relationship between occupancy of NO66 histone demethylase, and that of Osx as well as H3K4me3 or H3K36me3 [Sinha et al., 2010]. This supports the hypothesis that interactions of NO66 with the chromatin cause demethylation of these histone lysines for the maintenance of gene repression in the preosteoblast state. These studies open new insights into the regulation of osteoblast genes through epigenetic mechanisms involving histone methylation of the osteoblast-specific chromatin and provide clear evidence for a physiological role of NO66 in osteoblasts.

PHOSPHORYLATION OF Osx REGULATES ITS TRANSCRIPTIONAL ACTIVITY

Post-translational modification by phosphorylation elicited by signaling pathways is a key cellular event in controlling the activities of transcription factors during gene activation. Numerous studies have implicated for phosphorylation of Osx triggered by signaling pathways such as BM2 and MAPK in the regulation of Osx activity will be discussed here. Osx is phosphorylated by p38 MAPK at Ser-73 and Ser-77 to increase protein stability and to interact with transcriptional activators including p300 at the target *Bsp* and *Fmod* genes during osteoblast differentiation. BMP-2-induced Osx or ectopically expressed Osx exists as doublet polypeptide bands due to phosphorylation. Inhibition of p38 MAPK inhibits Osx expression at both mRNA and protein level [Ortuno et al., 2010]. Wang et al. [2011] showed that Spata4 (spermatogenesis associated 4 gene) interacts with Erk1/2 in osteoblasts and enhances phosphorylation of Erk1/2, which then phosphorylates Osx to increase protein stability and activity of Osx during activation of Osx target genes.

Based on converging evidence, phosphorylation of Osx is indispensible for Osx activity for expression of osteoblast genes. Akt is a serine/threonine-specific protein kinase that phosphorylates Osx at threonine residues present within Akt recognition element RXXS/T near the C terminus of Osx. BMP-2 treatment of C2C12 cells induces Osx protein in an Akt-dependent manner. Further, Akt stimulates Osx-dependent activation of the *Oc*, *Alp*, and *Bsp* promoters in transfection assays, indicating that phophorylation by Akt increases protein stability and Osx activity during osteoblast differentiation [Choi et al., 2011]. Control of Osx expression and the transcriptional activity of Osx are outlined in the figure.

CONCLUDING REMARKS

In this review, we have compiled many of the important research findings in genetic and molecular control of Osx in bone and tooth formation. Osx is a major effector and essential for activation of bone-specific genes that support bone formation in embryos and adults. Runx2 is a critical in formation of precursor osteoblasts from mesenchymes and Osx is a critical for differentiation of *Runx2*-expressing precursors into mature and functional osteoblasts. Since Osx is a classic osteoblast marker, the question remains to be addressed whether Osx once expressed takes control of transcriptional program of osteoblast target genes independently of Runx2 function. Animal models and cell culture studies indicate the potential for therapeutic use of Osx in bone injury repair. The published literature we have reviewed gives a brief assessment of Osx's role in control of bone formation, but unlike Runx2 which has been extensively characterized till date, we do not have explicit knowledge of the mechanisms underlying Osx expression or the regulation of Osx activity, which is essential for osteoblast formation. A list of suggested articles on detailed study of Osx is included in this review for the reader (Table I). Osx and its

TABLE I. Following Publications Are Suggested for Further Information on Osterix Studies

Description of studies	Experimental models	Authors
Discovery and function of Osx in osteoblast differentiation and bone formation during embryonic development	Mouse KO studies	Nakashima et al. [2002]
Role of Osx in bone homeostasis in adult and in formation of osteocytes	Conditional mouse KO	Zhou et al. [2010]
Frameshift mutation in *OSX* gene	Osteogenesis imperfecta	Lapunzina et al. [2010]
Function of Osx in cementogenesis (tooth formation)	Conditional mouse KO	Cao et al. [2012]
Expression patterns of Osx during tooth formation	Mouse studies	Hirata et al. [2009]
Osx-mediated inhibition in differentiation and maturation of chondrocytes	Mouse and cell culture	Kaback et al. [2008]
Runx2 independent activation of Osx through Dlx5	Cell culture studies	Lee et al. [2003]
Runx2-mediated activation of Osx	Cell culture studies	Nishio et al. [2006]
Requirement of precursor osteoblast (Osx^{+}) in primary ossification as well as in bone healing process	Mouse studies using lineage tracing	Maes et al. [2010]
ER-stress response in activation of Osx during osteoblast differentiation	Cell culture studies	Tohmonda et al. [2011]
Function of Osx in bone-related injuries	Mouse studies	Tu et al. [2006, 2007]
Inhibition of bone formation by p53 through negative regulation of Osx	Mouse KO studies	Wang et al. [2006]
Inhibition of osteoblast differentiation by NO66 histone demethylase through interactions with Osx	Cell culture studies	Sinha et al. [2010]
p38-mediated Osx phosphorylation	Cell culture studies	Ortuno et al. [2010]
BMP-2 dependent and independent activation of Osx through MAPK	Cell culture studies	Celil et al. [2005] and Celil and Campbell [2005]
Akt and Erk1/2 dependent Osx activity	Cell culture studies	Choi et al. [2011]
mir-93 controls Osx protein	Cell culture studies	Yang et al. [2012]

interactions with other proteins and post-translational modifications by phosphorylation and methylation, signaling pathways, miRNAs, and DNA methylation, any and all of which may regulate Osx expression and activity, should be considered for future work to better understand how Osx controls chromatin structure of its target genes during osteoblast differentiation. Finally, we leave a big question to our readers and for future studies—is Osx a slave of Runx2 or the master of skeletalogenesis?

ACKNOWLEDGMENTS

We extremely thank Dr. Benoit de Crombrugghe, Department of Genetics, M.D., Anderson Cancer Center, Houston, TX, for his unstinted support to us in conducting studies on Osterix, and his valuable suggestions during preparation of this review. Author's work was supported by grant AR049072 (B. de Crombrugghe) and AR061590 (KS).

REFERENCES

Baek WY, Lee MA, Jung JW, Kim SY, Akiyama H, de Crombrugghe B, Kim JE. 2009. Positive regulation of adult bone formation by osteoblast-specific transcription factor osterix. J Bone Miner Res 24:1055–1065.

Baek WY, de Crombrugghe B, Kim JE. 2010. Postnatally induced inactivation of Osterix in osteoblasts results in the reduction of bone formation and maintenance. Bone 46:920–928.

Benevolenskaya EV. 2007. Histone H3K4 demethylases are essential in development and differentiation. Biochem Cell Biol 85:435–443.

Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. 2006. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125:315–326.

Boot-Handford RP, Briggs MD. 2010. The unfolded protein response and its relevance to connective tissue diseases. Cell Tissue Res 339:197–211.

Bradley EW, McGee-Lawrence ME, Westendorf JJ. 2011. HDAC-mediated control of endochondral and intramembranous ossification. Crit Rev Eukaryot Gene Expr 21:101–113.

Cao Z, Zhang H, Zhou X, Han X, Ren Y, Gao T, Xiao Y, de Crombrugghe B, Somerman MJ, Feng JQ. 2012. Genetic evidence for the vital function of osterix in cementogenesis. J Bone Miner Res 27:1080–1092.

Celil AB, Campbell PG. 2005. BMP-2 and insulin-like growth factor-I mediate Osterix (Osx) expression in human mesenchymal stem cells via the MAPK and protein kinase D signaling pathways. J Biol Chem 280:31353–31359.

Celil AB, Hollinger JO, Campbell PG. 2005. Osx transcriptional regulation is mediated by additional pathways to BMP2/Smad signaling. J Cell Biochem 95:518–528.

Chen S, Gluhak-Heinrich J, Wang YH, Wu YM, Chuang HH, Chen L, Yuan GH, Dong J, Gay I, MacDougall M. 2009. Runx2, osx, and dspp in tooth development. J Dent Res 88:904–909.

Choi YH, Jeong HM, Jin YH, Li H, Yeo CY, Lee KY. 2011. Akt phosphorylates and regulates the osteogenic activity of Osterix. Biochem Biophys Res Commun 411:637–641.

Clarkin C, Olsen BR. 2010. On bone-forming cells and blood vessels in bone development. Cell metabolism 12:314–316.

Cloos PA, Christensen J, Agger K, Helin K. 2008. Erasing the methyl mark: histone demethylases at the center of cellular differentiation and disease. Genes Dev 22:1115–1140.

Delgado-Calle J, Sanudo C, Bolado A, Fernandez AF, Arozamena J, Pascual-Carra MA, Rodriguez-Rey JC, Fraga MF, Bonewald L, Riancho JA. 2012. DNA methylation contributes to the regulation of sclerostin expression in human osteocytes. J Bone Miner Res 27:926–937.

Eilbracht J, Reichenzeller M, Hergt M, Schnolzer M, Heid H, Stohr M, Franke WW, Schmidt-Zachmann MS. 2004. NO66, a highly conserved dual location protein in the nucleolus and in a special type of synchronously replicating chromatin. Mol Biol Cell 15:1816–1832.

Eskildsen T, Taipaleenmaki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, Bak M, Kauppinen S, Kassem M. 2011. MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. Proc Natl Acad Sci USA 108:6139–6144.

Fang J, Hall BK. 1997. Chondrogenic cell differentiation from membrane bone periostea. Anat Embryol (Berl) 196:349–362.

Franceschi RT. 2005. Biological approaches to bone regeneration by gene therapy. J Dent Res 84:1093–1103.

Goettsch C, Rauner M, Pacyna N, Hempel U, Bornstein SR, Hofbauer LC. 2011. miR-125b regulates calcification of vascular smooth muscle cells. Am J Pathol 179:1594–1600.

Hassan MQ, Saini S, Gordon JA, van Wijnen AJ, Montecino M, Stein JL, Stein GS, Lian JB. 2009. Molecular switches involving homeodomain proteins, HOXA10 and RUNX2 regulate osteoblastogenesis. Cells Tissues Organs 189:122–125.

Hatta M, Yoshimura Y, Deyama Y, Fukamizu A, Suzuki K. 2006. Molecular characterization of the zinc finger transcription factor, Osterix. Int J Mol Med 17:425–430.

Hirata A, Sugahara T, Nakamura H. 2009. Localization of runx2, osterix, and osteopontin in tooth root formation in rat molars. J Histochem Cytochem 57:397–403.

Huang W, Yang S, Shao J, Li YP. 2007. Signaling and transcriptional regulation in osteoblast commitment and differentiation. Front Biosci 12:3068–3092.

Hupkes M, van Someren EP, Middelkamp SH, Piek E, van Zoelen EJ, Dechering KJ. 2011. DNA methylation restricts spontaneous multi-lineage differentiation of mesenchymal progenitor cells, but is stable during growth factor-induced terminal differentiation. Biochim Biophys Acta 1813:839–849.

Jadlowiec J, Koch H, Zhang X, Campbell PG, Seyedain M, Sfeir C. 2004. Phosphophoryn regulates the gene expression and differentiation of NIH3T3, MC3 T3-E1, and human mesenchymal stem cells via the integrin/MAPK signaling pathway. J Biol Chem 279:53323–53330.

Jensen ED, Nair AK, Westendorf JJ. 2007. Histone deacetylase co-repressor complex control of Runx2 and bone formation. Crit Rev Eukaryot Gene Expr 17:187–196.

Jensen ED, Schroeder TM, Bailey J, Gopalakrishnan R, Westendorf JJ. 2008. Histone deacetylase 7 associates with Runx2 and represses its activity during osteoblast maturation in a deacetylation-independent manner. J Bone Miner Res 23:361–372.

Kaback LA, Soung do Y, Naik A, Smith N, Schwarz EM, O'Keefe RJ, Drissi H. 2008. Osterix/Sp7 regulates mesenchymal stem cell mediated endochondral ossification. J Cell Physiol 214:173–182.

Kang JS, Alliston T, Delston R, Derynck R. 2005. Repression of Runx2 function by TGF-beta through recruitment of class II histone deacetylases by Smad3. Embo J 24:2543–2555.

Karsenty G. 2001. Minireview: transcriptional control of osteoblast differentiation. Endocrinology 142:2731–2733.

Karsenty G. 2008. Transcriptional control of skeletogenesis. Ann Rev Genomics Hum Genet 9:183–196.

Klose RJ, Kallin EM, Zhang Y. 2006. JmjC-domain-containing proteins and histone demethylation. Nat Rev Genet 7:715–727.

Koga T, Matsui Y, Asagiri M, Kodama T, de Crombrugghe B, Nakashima K, Takayanagi H. 2005. NFAT and Osterix cooperatively regulate bone formation. Nat Med 11:880–885.

Lapunzina P, Aglan M, Temtamy S, Caparros-Martin JA, Valencia M, Leton R, Martinez-Glez V, Elhossini R, Amr K, Vilaboa N, Ruiz-Perez VL. 2010. Identification of a frameshift mutation in Osterix in a patient with recessive osteogenesis imperfecta. Am J Hum Genet 87:110–114.

Lee MH, Kwon TG, Park HS, Wozney JM, Ryoo HM. 2003. BMP-2-induced Osterix expression is mediated by Dlx5 but is independent of Runx2. Biochem Biophys Res Commun 309:689–694.

Lian JB, Stein GS, van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, Zhang Y. 2012. MicroRNA control of bone formation and homeostasis. Nat Rev Endocrinol 8:212–227.

Liu H, Li B. 2010. p53 control of bone remodeling. J Cell Biochem 111:529–534.

Lu X, Beck GR, Jr, Gilbert LC, Camalier CE, Bateman NW, Hood BL, Conrads TP, Kern MJ, You S, Chen H, Nanes MS. 2011. Identification of the homeobox protein Prx1 (MHox, Prrx-1) as a regulator of osterix expression and mediator of tumor necrosis factor alpha action in osteoblast differentiation. J Bone Miner Res 26:209–219.

Maes C, Kobayashi T, Selig MK, Torrekens S, Roth SI, Mackem S, Carmeliet G, Kronenberg HM. 2010. Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. Dev Cell 19:329–344.

Martin C, Zhang Y. 2005. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol 6:838–849.

Minard ME, Jain AK, Barton MC. 2009. Analysis of epigenetic alterations to chromatin during development. Genesis 47:559–572.

Murakami T, Saito A, Hino S, Kondo S, Kanemoto S, Chihara K, Sekiya H, Tsumagari K, Ochiai K, Yoshinaga K, Saitoh M, Nishimura R, Yoneda T, Kou I, Furuichi T, Ikegawa S, Ikawa M, Okabe M, Wanaka A, Imaizumi K. 2009. Signalling mediated by the endoplasmic reticulum stress transducer OASIS is involved in bone formation. Nat Cell Biol 11:1205–1211.

Nakashima K, de Crombrugghe B. 2003. Transcriptional mechanisms in osteoblast differentiation and bone formation. Trends Genet 19:458–466.

Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B. 2002. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29.

Nishimura R, Wakabayashi M, Hata K, Matsubara T, Honma S, Wakisaka S, Kiyonari H, Shioi G, Yamaguchi A, Tsumaki N, Akiyama H, Yoneda T. 2012. Osterix regulates calcification and degradation of chondrogenic matrices through matrix metalloproteinase 13 (MMP13) expression in association with transcription factor Runx2 during endochondral ossification. J Biol Chem 287:33179–33190.

Nishio Y, Dong Y, Paris M, O'Keefe RJ, Schwarz EM, Drissi H. 2006. Runx2-mediated regulation of the zinc finger Osterix/Sp7 gene. Gene 372:62–70.

OLee JY, Lee YM, Kim MJ, Choi JY, Park EK, Kim SY, Lee SP, Yang JS, Kim DS. 2006. Methylation of the mouse DIx5 and Osx gene promoters regulates cell type-specific gene expression. Mol Cells 22:182–188.

Ong CT, Corces VG. 2012. Enhancers: emerging roles in cell fate specification. EMBO Rep 13:423–430.

Ortuno MJ, Ruiz-Gaspa S, Rodriguez-Carballo E, Susperregui AR, Bartrons R, Rosa JL, Ventura F. 2010. p38 regulates expression of osteoblast-specific genes by phosphorylation of osterix. J Biol Chem 285:31985–31994.

Park SJ, Kim KJ, Kim WU, Oh IH, Cho CS. 2012. Involvement of endoplasmic reticulum stress in homocysteine-induced apoptosis of osteoblastic cells. J Bone Miner Metab 30:474–484.

Rodda SJ, McMahon AP. 2006. Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. Development 133:3231–3244.

Schaap-Oziemlak AM, Raymakers RA, Bergevoet SM, Gilissen C, Jansen BJ, Adema GJ, Kogler G, le Sage C, Agami R, van der Reijden BA, Jansen JH. 2010. MicroRNA hsa-miR-135b regulates mineralization in osteogenic differentiation of human unrestricted somatic stem cells. Stem Cells Dev 19:877–885.

Sinha KM, Yasuda H, Coombes MM, Dent SY, de Crombrugghe B. 2010. Regulation of the osteoblast-specific transcription factor Osterix by NO66, a Jumonji family histone demethylase. EMBO J 29:68–79.

Timpson NJ, Tobias JH, Richards JB, Soranzo N, Duncan EL, Sims AM, Whittaker P, Kumanduri V, Zhai G, Glaser B, Eisman J, Jones G, Nicholson G, Prince R, Seeman E, Spector TD, Brown MA, Peltonen L, Smith GD, Deloukas P, Evans DM. 2009. Common variants in the region around Osterix are associated with bone mineral density and growth in childhood. Hum Mol Genet 18:1510–1517.

Tohmonda T, Miyauchi Y, Ghosh R, Yoda M, Uchikawa S, Takito J, Morioka H, Nakamura M, Iwawaki T, Chiba K, Toyama Y, Urano F, Horiuchi K. 2011. The IRE1alpha-XBP1 pathway is essential for osteoblast differentiation through promoting transcription of Osterix. EMBO Rep 12:451–457.

Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, Zhang Y. 2006. Histone demethylation by a family of JmjC domain-containing proteins. Nature 439:811–816.

Tu Q, Valverde P, Chen J. 2006. Osterix enhances proliferation and osteogenic potential of bone marrow stromal cells. Biochem Biophys Res Commun 341:1257–1265.

Tu Q, Valverde P, Li S, Zhang J, Yang P, Chen J. 2007. Osterix overexpression in mesenchymal stem cells stimulates healing of critical-sized defects in murine calvarial bone. Tissue Eng 13:2431–2440.

Wang X, Kua HY, Hu Y, Guo K, Zeng Q, Wu Q, Ng HH, Karsenty G, de Crombrugghe B, Yeh J, Li B. 2006. p53 functions as a negative regulator of osteoblastogenesis, osteoblast-dependent osteoclastogenesis, and bone remodeling. J Cell Biol 172:115–125.

Wang X, Harimoto K, Liu J, Guo J, Hinshaw S, Chang Z, Wang Z. 2011. Spata4 promotes osteoblast differentiation through Erk-activated Runx2 pathway. J Bone Miner Res 26:1964–1973.

Xing W, Pourteymoor S, Mohan S. 2011. Ascorbic acid regulates osterix expression in osteoblasts by activation of prolyl hydroxylase and ubiquitination-mediated proteosomal degradation pathway. Physiol Genomics 43:749–757.

Xu B, Zhang J, Brewer E, Tu Q, Yu L, Tang J, Krebsbach P, Wieland M, Chen J. 2009. Osterix enhances BMSC-associated osseointegration of implants. J Dent Res 88:1003–1007.

Yang X, Karsenty G. 2002. Transcription factors in bone: developmental and pathological aspects. Trends Mol Med 8:340–345.

Yang X, Matsuda K, Bialek P, Jacquot S, Masuoka HC, Schinke T, Li L, Brancorsini S, Sassone-Corsi P, Townes TM, Hanauer A, Karsenty G. 2004. ATF4 is a substrate of RSK2 and an essential regulator of osteoblast biology; implication for Coffin-Lowry syndrome. Cell 117:387–398.

Yang L, Cheng P, Chen C, He HB, Xie GQ, Zhou HD, Xie H, Wu XP, Luo XH. 2012. miR-93/Sp7 function loop mediates osteoblast mineralization. J Bone Miner Res 27:1598–1606.

Yoshida CA, Komori H, Maruyama Z, Miyazaki T, Kawasaki K, Furuichi T, Fukuyama R, Mori M, Yamana K, Nakamura K, Liu W, Toyosawa S, Moriishi T, Kawaguchi H, Takada K, Komori T. 2012. SP7 inhibits osteoblast differentiation at a late stage in mice. PLoS ONE 7:e32364.

Zhang JF, Fu WM, He ML, Wang H, Wang WM, Yu SC, Bian XW, Zhou J, Lin MC, Lu G, Poon WS, Kung HF. 2011. MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix. Mol Biol Cell 22:3955–3961.

Zhao C, Hosoya A, Kurita H, Hu T, Hiraga T, Ninomiya T, Yoshiba K, Yoshiba N, Takahashi M, Kurashina K, Ozawa H, Nakamura H. 2007. Immunohistochemical study of hard tissue formation in the rat pulp cavity after tooth replantation. Arch Oral Biol 52:945–953.

Zhao J, Bradfield JP, Li M, Zhang H, Mentch FD, Wang K, Sleiman PM, Kim CE, Glessner JT, Frackelton EC, Chiavacci RM, Berkowitz RI, Zemel BS,

Hakonarson H, Grant SF. 2011. BMD-associated variation at the Osterix locus is correlated with childhood obesity in females. Obesity (Silver Spring) 19:1311–1314.

Zhou X, Zhang Z, Feng JQ, Dusevich VM, Sinha K, Zhang H, Darnay BG, de Crombrugghe B. 2010. Multiple functions of Osterix are required for bone growth and homeostasis in postnatal mice. Proc Natl Acad Sci USA 107:12919–12924.

Zhu J, Shimizu E, Zhang X, Partridge NC, Qin L. 2011. EGFR signaling suppresses osteoblast differentiation and inhibits expression of master osteoblastic transcription factors Runx2 and Osterix. J Cell Biochem 112:1749–1760.

Zou L, Zou X, Li H, Mygind T, Zeng Y, Lu N, Bunger C. 2006. Molecular mechanism of osteochondroprogenitor fate determination during bone formation. Adv Exp Med Biol 585:431–441.