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Transcriptional regulation of bone formation

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1. ABSTRACT

Bone formation in vertebrates depends on the proliferation and differentiation of chondrocytes and osteoblasts, which are derived from a mesenchymal precursor cells and are required for skeletal development. This process relies on many transcription factor genes that have functions in bone cell proliferation and differentiation. Over the years, various transcription factors that play essential roles for skeletal formation have been identified in *in vivo* and/or *in vitro* molecular and genetic studies. In regulation of bone formation, signaling pathways with these factors have also been established. Major transcription factors including Sox9, Runx2, and Osterix, which are active in chondrocytes or osteoblasts or even in both cells, are indispensable for chondrocyte and/or osteoblast differentiation. Here, the transcriptional regulation of these genes for bone formation will be reviewed on the basis of *in vivo* mouse models.

2. INTRODUCTION

Bone is a living dynamic connective tissue that is constantly reconstructed by remodeling throughout life. The function of bone includes movement, support, and protection of internal organs of the body. Bone is also important to produce blood cells and reserve minerals. Many genes encoding either growth factors or transcription factors that are involved in patterning of various skeletal elements have been identified. The current understanding of the molecular and cellular functions for transcription factors in skeletal development is the result of experimental animal studies using transgenic and/or knockout mouse models. Indeed, these *in vivo* molecular and genetic studies in mice have provided new insights as well as mechanisms into the transcriptional regulation of bone formation. To date, the basic and physiological aspects of bone formation have been reviewed from *in vivo* and *in vitro* perspectives (1-3). Here, this review will more focus on major

transcription factors regulating bone formation based on updated *in vivo* mouse models.

3. BONE FORMATION

The skeleton is a complex organ composed of cartilage and bone that contains various chondrocytes, osteoblastic cells, and osteoclasts (4, 5). During skeletal development, bones are formed by two distinct pathways - intramembranous and endochondral ossification. Bone tissues in intramembranous ossification are formed by mesenchymal cells that condense to become functional osteoblasts directly without cartilage formation. This process occurs in most flat bones including skull bones and some irregular bones. Unlike intramembranous ossification, endochondral ossification is a process in which bone formation begins from a cartilage template model that is later replaced by osteoblastc cells with infiltrating blood vessels, followed by the formation of bone (5). Most bones in the skeleton develop through this process.

3.1. Chondrogenesis

Chondrogenesis, which is the earliest process of skeletal development, involves mesenchymal cell recruitment and migration, condensation of progenitors, and chondrocyte differentiation and maturation. This process results in the formation of cartilage intermediate and leads to endochondral ossification (5). Chondrocytes, which are needed for chondrogenesis, are the first skeleton-specific cells to appear during skeletal development and lead to bone formation. During mouse development, mesenchymal condensation is formed as a template of the future skeleton at embryonic day 10.5 (E10.5). These mesenchymal cells in the center of the area of condensation differentiate into proliferating chondrocytes that produce type II collagen and aggrecan (6-8). However, peripheral cells at the mesenchymal condensations do not differentiate into chondrocytes, but instead form the perichondrium to influence chondrocyte maturation (6-8). At E13.5, proliferating chondrocytes begin to be elongated, which lead to the formation of prehypertrophic chondrocytes. The prehypertrophic chondrocytes that still produce type II collagen then mature into hypertrophic chondrocytes that produce type X collagen (8, 9). When chondrocytes become hypertrophic, cells of perichondrium start to express runt-related transcription factor 2 (Runx2), which is a key regulator of osteoblast differentiation, to form bone (10). This is followed by the creation of bone collar, which is a structure that prefigures the future cortical bone (6-8, 11). The connective tissue around the bone collar, which is previously a perichondrium, is now called the periosteum. ECM mineralization surrounding the hypertrophic chondrocytes occurs via the secretion of type X collagen and vascular invasion from the bone collar arises through vascular endothelial growth factor (VEGF) (12). With invading blood vessels, hypertrophic chondrocytes undergo apoptosis and are displaced by osteoblastic cells. In this process, which occurs centrifugally, the primary ossification centers develop in the diaphysis of long bones during fetal life and secondary ossification centers develop in the epiphysis after birth. The growth plate, which is arranged by proliferating and hypertrophic chondrocytes into a columnar pattern, is located between the epiphysis and the methaphysis at each end of the expanding bone, thereby leading to the longitudinal growth of the skeleton (1, 11). In human, this growth plate reduces to an epiphyseal line after puberty, and the long bone no longer grows in length.

Chondrocyte differentiation is regulated by a large number of genes. Transcription factor Sox9 is a member of the sex determining factor (SRY)-related gene family (13). During mouse development, Sox9 is expressed in mesenchymal precursor cells, which give rise to chondrocytes as well as ostseoblasts, and its expression overlaps with that of type II collagen (Col2a1), a major matrix protein in cartilage (14). Sox9 is required for chondrocyte differentiation, together with L-Sox5 and Sox6 (13, 15). In Sox9-inactivated mutants before mesenchymal condensations occurs, a complete absence of both cartilage and bone is observed. Sox9 inactivation after mesenchymal condensations results in severe chondrodysplasia, which is shown in Sox5 and Sox6 double-null mutants (16). Sox9-overexpressed mice in chondrocytes display chondrodysplasia by the inhibition of chondrocyte proliferation (17). Indian hedgehog (Ihh) produced by prehypertrophic chondrocytes is a master regulator of endochondral bone formation that coordinates chondrocyte proliferation and maturation and osteoblast differentiation (2, 11). Parathyroid hormone-related protein (PTHrP) is a protein secreted by perichondrial cells and proliferating chondrocytes. PTHrP acts on receptors on proliferating chondrocytes to maintain chondrocyte proliferation and to delay Ihh production (2, 11). In growth plate, eventually, these paracrine factors together control chondrocyte differentiation through a reciprocal negative-feedback mechanism. When chondrocytes proliferation stimulated by PTHrP is completed, chondrocytes produce Ihh. Even though mechanisms are still unclear, Ihh stimulates PTHrP synthesis at the periarticular region of long bone through a feedback loop (11). PTHrP regulates the differentiation and proliferation of growth plate chondrocytes through the Gli3 transcriptional activity in a complex feedback loop (18, 19). Ihh-deficient mice exhibit small cartilage elements due to decreased chondrocyte proliferation and maturation (20). Overexpressed Ihh increases the expression of perichondrial PTHrP, which keeps chondrocytes in the proliferating state and inhibits the differentiation into hypertrophic chondrocytes, indicating that Ihh regulates chondrocyte differentiation through a negative-feedback (18, 21, 22). In PTHrP-deficient mice, the proliferation of chondrocytes decreases and they become hypertrophic, which lead to premature chondrocytes and accelerated bone formation (23). Conversely, overexpression of PTHrP in proliferating chondrocytes results in dwarfism with short limbs and delayed endochondral ossification (24). The overexpression of constitutively active PTH/PTHrP receptor in growth plate decreases conversion of proliferating chondrocytes into hypertrophic chondrocytes (25). Furthermore, this overexpression rescues postnatal growth plate abnormalities of PTHrP-deficient mice and/or Ihh-lacking mice (25, 26). In postnatal period, the region of periarticular cartilage is not proper for PTHrP synthesis due to the distance between the epiphyseal growth plate and the

periarticular surface, and the production of Ihh is not observed in growth plate, suggesting that other signals may regulate chondrocyte differentiation in adolescence (11, 27). PTHrP expression in chondrocytes is also controlled by the signaling of TGF-β which action is mediated by Smad3 (28, 29). Smad3-deficient mice exhibit the disorganized growth plate and premature hypertrophy of articular chondrocytes after birth, indicating that TGF-β signaling through Smad3 is required for postnatal bone growth (30).

3.2. Osteogenesis

In osteogenesis for bone formation, pluripotent mesenchymal stem cells have the potential to differentiate into various lineages including cartilage, bone, adipose, and muscle (31). A commitment of mesenchymal cells into osteoprogenitor cells is controlled by many types of inducers including the transforming growth factor (TGF)-β superfamily and bone morphogenetic proteins (BMPs) (32, 33). Osteoprogenitor cells are multipotent cells in bone marrow and periosteum that differentiate into preosteoblasts and then mature osteoblasts lining the bone surfaces (1-3, 34). In the presence of type I collagen (Col1a1) and alkaline phosphatase, proliferation and maturation of the osteoblastic lineage begin in preosteoblastic cells that are in the early stage of development and not yet capable of synthesizing bone matrix (2, 5, 34). Osteoblasts are then induced through the expression of osteoblast-specific transcription factors Runx2 and/or Osterix (Osx) in preosteoblasts (34-37). Osteoblasts are the well-differentiated product of osteoprogenitor cells and mature active cuboidal shaped cells located on the bone surfaces (33, 34). These cells are functionally responsible for bone formation that directs the deposition and calcification of bone matrix. When osteoblast differentiation is finished, osteoblasts can be bone lining cells located on a quiescent bone surface for protection or osteocytes enclosed in the mineralized bone matrix. Osteocytes are the terminally differentiated osteoblasts that support bone structure and modify their microenvironment. Individual osteocytes are connected with other osteocytes through dendritic processes to enable communication with each other (38). Osteocytes also function as a signaling network to regulate mineralization and calcium/phosphate homeostasis in bone (38). During osteogenic differentiation, each maturation stage is demonstrated by the expression of each marker gene. For example, alkaline phosphatase and osteocalcin are marker genes during the early stage and late stage of differentiation, respectively.

Various signaling molecules such as growth factors, hormones, and transcription factors are involved in osteoblast differentiation from osteoprogenitor cells (2, 3, 35). In particular, the regulation by transcription factors is essential to osteogenic differentiation. Canonical Wnt signaling via β-catenin plays an essential regulatory role in osteoblast differentiation *in vivo* (39). Msx2, a homeodomain transcription factor, promotes osteoblast proliferation and differentiation in mesenchymal progenitor cells (40, 41). In particular, Runx2, Osx, and activating transcription factor 4 (ATF4) are major transcription factors required for bone formation. These factors are reviewed in detail in the next chapter.

3.3. Bone modeling and remodeling

The major methods of bone structural adaptation are modeling and remodeling (42). These two processes are not very different at the cellular level based on the separate actions of osteoclasts and osteoblasts. Osteoclasts, which are the major bone resorbing cells, are generated from progenitors of monocytes/macrophages that differentiate into mononuclear precursor (preosteoclast) cells under the control of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kB ligand (RANKL) (43). RANKL secreted from marrow stromal cells and osteoblasts links to its receptor, RANK, on the surface of preosteoclast cells, inducing their differentiation and fusion for multinucleated and functional osteoclasts (44). In addition to RANKL, several differentiating factors including interleukin-1, tumor necrosis factor (TNF), parathyroid hormone (PTH), and vitamin D are also involved in osteoclast maturation (43, 45). Osteoblasts secrete a soluble decoy receptor, osteoprotegerin (OPG), as well as RANKL. OPG blocks the interaction between RANK and RANKL by binding to RANKL, which prevents osteoclast differentiation and activation (45). This indicates that the balance between RANKL and OPG from osteoblasts is important to determine osteoclast formation and activity.

Bone modeling leads to the reshaping of bone through the independent action of osteoblasts and osteoclasts. During initial bone formation in the embryonic stage, large changes in the bone shape are needed. In growing bone, the deposition of calcified tissue lead to increased thickness of long bones by appositional growth and increased length at the epiphyseal growth plates by longitudinal growth. The process of bone growth occurs continuously through repeated cycles of the partial resorption of preformed tissue and the replacement with new bone (34, 42, 46). Finally, in bone modeling, bone resorption and formation occur on different surfaces, resulting in large changes in bone structure. Compared to bone modeling, bone remodeling is a vital physiological process that occurs concurrently at multiple locations of the skeleton (42). While modeling involves independent actions of both cells, bone remodeling involves the sequential action of osteoblasts and osteoclasts. Unlike modeling, remodeling cannot cause large changes in bone structure at a given site. Remodeling is needed to maintain the skeletal structure and to assist its metabolic functions as a reservoir of calcium and phosphorus (46). This process takes place to the stimulation of cavity formation by bone resorption, and the subsequent filling of these cavities by new bone formation. During remodeling, the levels of cellular activity are equal in the coupling of bone resorption and formation so that bone turnover is balanced. A decrease in osteoclast numbers or bone resorption activity should be compensated by a decrease in osteoblast and bone formation, resulting in the maintenance of bone mass via a decreased turnover rate. Similarly, an increase in osteoclast activity should be associated with an increase in osteoblasts and bone formation, resulting in maintenance of bone mass

with a high turnover. Multiple examples of bone diseases result from unbalanced function between bone resorption and formation. Osteopetrosis results from an excess of bone formation compared to resorption, whereas a greater bone resorption than formation results in osteoporosis (47). Hyperparathyroidism and hyperthyroidism are bone disorders in which there is a markedly increased bone turnover (46, 47). Paget's disease of bone is a chronic skeletal disorder with abnormal turnover as a result of irregular bone resorption and formation (46).

4. MAJOR TRANSCRIPTIONAL CONTROL OF BONE FORMATION BY OSTEOBLASTS

Skeletal lineage is controlled by various transcription factors that are temporally expressed. Among them, three major transcription factors that are predominantly expressed in osteoblasts are Runx2 (34-36, 48), Osx (34, 37), and ATF4 (49). During bone formation, Runx2 is strongly expressed during the early stage in which mesenchymal cells are initiated to condense, after which they proceed into the chondrogenic or osteogenic lineages (10, 50). The expression of Runx2 remains in the committed osteoblastic lineage and is suppressed to enable immature osteoblasts to become fully mature osteoblasts (10, 48). Osx is an osteoblast-specific transcription factor that acts downstream of Runx2 to commit osteochondroprogenitor cells to the osteoblast lineage (34, 37). Subsequently, another bone-related transcription factor, ATF4, is a critical substrate of RSK2 that is required for the terminal differentiation of osteoblasts and bone development (49). Based on previous genetic studies, we provide here a better understanding of the cooperative interactions and network that occurs between major transcription factors involved in bone cell differentiation during skeletal development.

4.1. Regulation of osteoblast differentiation by Runx2

Runx2 is a member of the Runx family of transcription factors that has a highly conserved runt domain, which is a protein motif of 128 amino acids. Runx2 was first identified as a transactivator that binds to osteoblast-specific cis-acting element 2 (OSE2), which leads to activation of the expression of osteocalcin (10, 51). The expression of Runx2 is specifically observed in chondrocytes, osteoblasts, and other cell types of mesenchymal origin (10). During mouse development, Runx2 expression is initiated in cells of the mesenchymal condensation starting at E10.5 and detectable in osteochondroprogenitor cells of the perichondrium and bone collar, which have the potential to become osteoblasts (10). Next, Runx2 expression is restricted in osteoblastic cells but not in differentiated chondrocytes (10, 51). Therefore, Runx2 is required to regulate the expression of bone matrix proteins during chondrogenesis and at an early stage of osteoblast differentiation and bone formation (10, 48).

In vivo functions of Runx2 have been demonstrated in Runx2 knockout and/or overexpressed mouse models. Runx2 null mutants die shortly after birth with a complete absence of osteogenesis as well as delayed chondrocyte differentiation, consequently lacking of intramembranous and endochondral ossification (52, 53). Runx2 overexpression in chondrocytes under the control of the chondrocyte-specific type II collagen (Col2a1) promoter shows ectopic chondrocyte hypertrophy, induces endochondral ossification, and finally fails to form permanent cartilage during endochondral ossification (54, 55). Runx2-overexpressed mice in osteoblasts using type I collagen (Col1a1) promoter exhibit an osteopenic skeleton with impaired matrix production and mineralization, and multiple fractures demonstrating that Runx2 inhibits osteoblast differentiation at a late stage (56). The important role of Runx2 in skeletal development is further established by a hereditary autosomal dominant congenital disorder, cleidocranial dysplasia (CCD), which is caused by haploinsufficiency of Runx2. This phenotype, which has defects in the development of bones, is also observed in Runx2 heterozygous mice as well as humans with Runx2 mutations (57). Overall, these results suggest that Runx2 is necessary for chondrocyte and osteoblast differentiation and bone formation during skeletal development.

During skeletogenesis, numerous transcriptional co-factors regulate Runx2 expression and its activity (Figure 1). Twist-1 and -2 are transcription factors that have the structure of a basic helix-loop-helix protein and are expressed *in vivo* in Runx2-expressing cells throughout the skeleton during early development (58). These transcription factors bind directly to the runt DNA binding domain of Runx2 and decrease the ability of Runx2 to bind to DNA (58, 59). Twist-1 or -2 deficiency leads to the premature osteoblast differentiation that is observed in craniosynostosis, whereas its overexpression inhibits osteoblast differentiation during early skeletogenesis of Runx2-expressing cells (58). The zinc-finger adaptor protein Schnurri-3 (Shn3) has been shown to function in the immune system (60). Mice lacking Shn3 show severe osteosclerosis with increased bone mass due to the accumulation of Runx2 in Shn3-deficient osteoblasts, indicating that Shn3 regulates osteoblast function and bone formation by controlling the protein levels of Runx2 (61). Another nuclear matrix protein, SATB2, is expressed at sites of bone formation as well as in the brain cortex (62). Satb2-deficient mice exhibit craniofacial abnormality and the inhibition of osteoblast differentiation and function. Additionally, synergistic reduction in bone formation is observed in Satb2/Runx2 double heterozygotes compared to Satb2 heterozygotes, indicating that SATB2 enhances Runx2 functions for osteoblast differentiation during skeletal development (62). The function and activity of Runx2 in osteoblasts are also regulated by the signal transducer and activator of transcription 1 (Stat1), histone deacetylases (HDACs), and other transcription factor partners. Stat1-deficiency efficiently undergoes Runx2 translocation into the nucleus and finally exhibits an increased bone mass as a result of excessive osteoblast differentiation (63). The suppression of co-repressor HDAC3, which interacts with Runx2, accelerates the mineralization of bone matrix and the expression of osteogenic marker genes in osteoblastic cells (64). Deficiency and overexpression of another co-repressor, HDAC4, in mice result in premature ossification by the

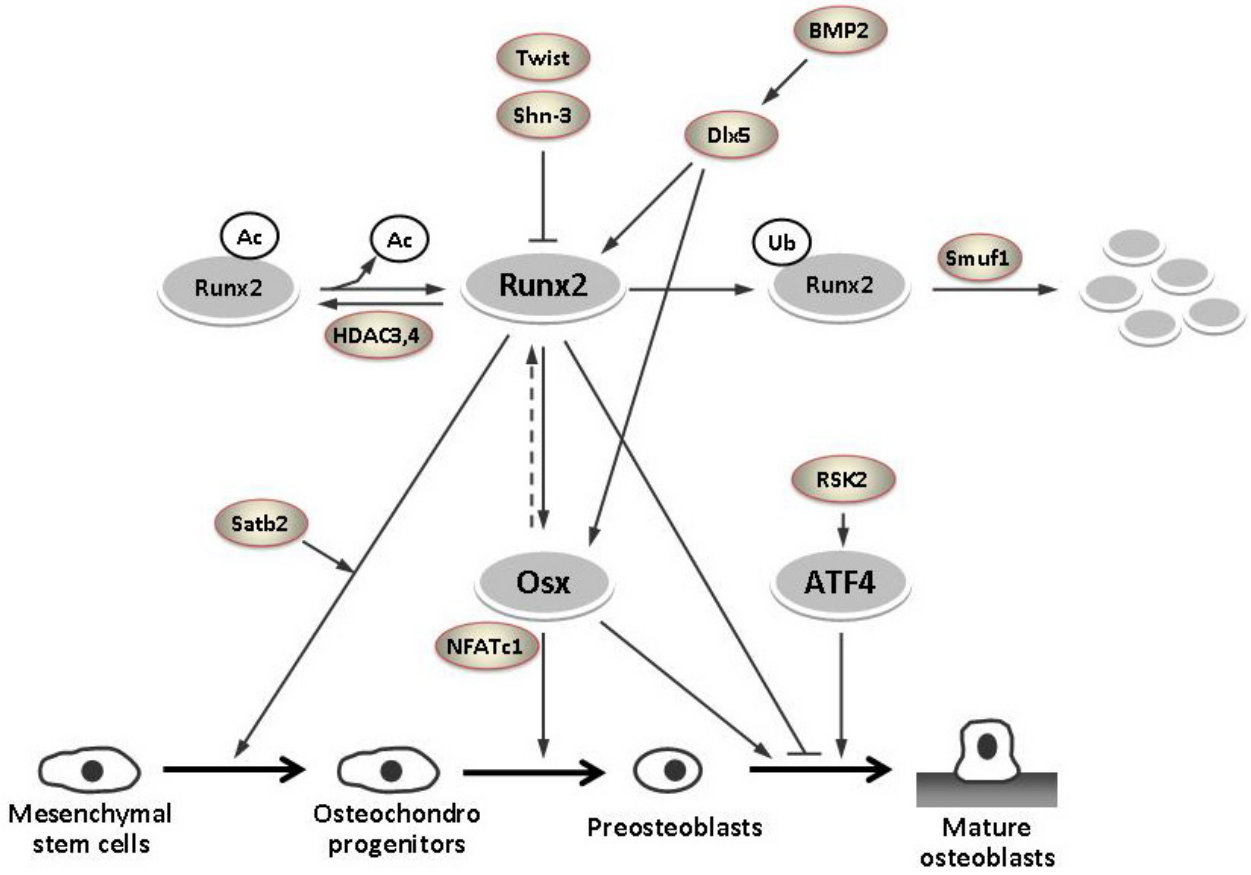

Figure 1. Regulation of osteoblast differentiation by transcription factors. The major transcription factors Runx2, Osx, and ATF4, which are activated and regulated by other important transcription factors, control the differentiation and maturation into the osteoblastic lineage starting from mesenchymal stem cells. In osteoblast differentiation, Runx2 is essential for the osteogenic potential of uncommitted osteochondroprogenitors and Osx is required for the commitment of osteochondroprogenitors to preosteoblasts and mature osteoblasts.

ectopic and early onset chondrocyte hypertrophy and the inhibition of chondrocyte hypertrophy and differentiation, respectively, suggesting that Runx2 activity is controlled by HDAC4 to block Runx2 DNA binding in chondrocytes (65). The E3 ubiquitin ligase Smurf1, which mediates Runx2 degradation, suppresses osteoblast differentiation and bone formation when it is overexpressed and increases bone mass by its deficiency (66, 67).

4.2. Regulation of osteoblast differentiation by Osterix

Osterix (Osx) is a transcription factor that belongs to a member of the Krüppel-like family and consists of three C2H2-type zinc fingers DNA binding domain at the C-terminus. Osx is highly specific to osteoblasts *in vivo* and mainly expressed in osteoblast progenitor cells of all developing endochondral and intramembranous bones, suggesting that Osx is required for the differentiation of preosteoblasts into functional osteoblasts (37). During mouse development, Osx expression is first initiated in differentiating chondrocytes and the surrounding perichondrium at E13.5, after which it is restricted to the peripheral layers of endochondral skeletal elements. After that, the strong expression of Osx is observed in osteoblastic cells when compared to the weak expression in prehypertrophic chondrocytes. Osx expression is remained in the bone postnatally (37).

Osx-null mutants die in the immediate perinatal period due to the formation of a normal cartilage skeleton but a complete absence of all bone formation (37). Osx is not expressed in Runx2-deficient osteoblasts, whereas Runx2 is expressed in Osx-deficient osteoblasts, indicating that Osx acts downstream of Runx2. Conditional Osx knockout mice are used to further examine the *in vivo* function of Osx in growing or already formed bone (68). Osx expression in a conditional Osx mouse model is inactivated in all osteoblasts under the control of a 2.3-kb Col1a1 promoter using the Cre/loxP system (69, 70). First, Osx inactivation in osteoblasts of growing bones accumulates early-stage and immature osteoblasts with increased osteopontin but decreased osteocalcin expression, indicating that delayed osteoblast maturation and reduced osteoblast function leads to decreased bone formation in adult bone without Osx (69). In another study using the inducible Cre system, Osx is inactivated in osteoblasts of already formed bones after birth (70, 71). Although the

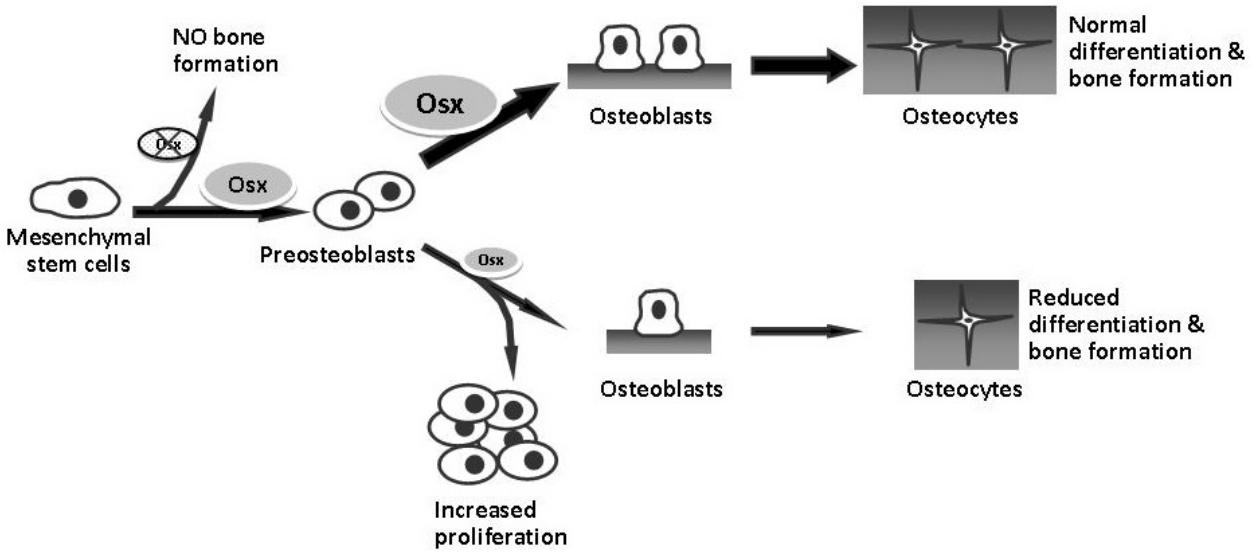

Figure 2. Schematic model for bone formation and maintenance governed by Osx expression. With a complete absence of Osx during skeletal development, no bone is formed. With the lack of Osx during skeletal growth, the differentiation of preosteoblasts into mature osteoblasts is reduced and the proliferation of preosteoblasts is increased instead, and then osteoblast function is decreased. This is followed by reduced bone formation.

increase in osteoblast proliferation and the accumulation of immature osteoblasts which are observed in previous results do not occur, Osx inactivation in osteoblasts during the postnatal period is sufficient to reduce bone formation with functional defects in osteoblasts (70). These two studies using conditional Osx knockout model may explain a functional difference of Osx in bone formation according to the time of its expression. Taken together, the *in vivo* results indicate that Osx is essential to the regulation of osteoblast function following bone maintenance and homeostasis during postnatal periods as well as developmental stages (Figure 2). Interestingly, the expression of Runx2 which is known to be an upstream gene is increased in osteoblast-specific Osx-inactivated adult mice (69). This suggests that Runx2 is not only an upstream gene of Osx, but is also regulated by activation of the unknown Osx-mediated genes or a negative feedback mechanism.

Recently, the regulation of Osx expression and activity by both Runx2-dependent and -independent mechanisms is reported (Figure 1). A nuclear factor of activated T cells NFATc, which is not essential for osteoblast differentiation, interacts with Osx and its complex binds to DNA and regulates the transcriptional activity of Osx (72). Overexpression of NFATc1 stimulates Osx-dependent activation of the Col1a1 promoter in primary osteoblasts, indicating that NFAT and Osx cooperatively control bone formation (72). Independent of the levels of Runx2 activity, Dlx5 induced by bone morphogenic protein-2 (BMP-2) binds to the specific BMP-responsive homeodomain element located in the proximal region of the Osx promoter and then activates osteogenic function of Osx (73). Osx expression is also upregulated by Smad signaling or by BMP-2 through Msx2, Runx2-independently, during osteoblast differentiation (74). Wnt/β-catenin signaling has been shown to play a critical role in controlling osteoblast proliferation and differentiation (39). The transcriptional activity of β-catenin in cell proliferation is inhibited by Osx, suggesting a possible mechanism for the inhibition of Osx in osteoblast proliferation (75). Another possible downstream target of Osx is dickkopf-1 (Dkk1), which is co-expressed with Osx in osteoblasts and upregulated by Osx overexpression. Osx binds to and activates Dkk1 promoter, demonstrating that Dkk1 may be a direct target of Osx (75). Jumonji C (JmjC)-domain containing histone demethylase NO66 interacts with Osx to inhibit the activation of Osx-mediated promoter and to control Osx-target genes in osteoblasts by modulating histone methylation states (76). Overexpression of T-box 3 containing a highly homologous DNA binding Tbx domain blocks bone mineralization and abolishes increased Runx2 and Osx expression during osteoblastic cell differentiation, indicating that it negatively regulates osteoblast differentiation by inhibiting the expression of Runx2 and Osx (77).

4.3. Regulation of osteoblast differentiation by ATF4

ATF4 is a basic leucine-zipper transcription factor that belongs to the ATF/CREB protein family (49, 78). During mouse development, ATF4 is dispensable for the early stages of skeletogenesis, but required for terminal differentiation of osteoblasts throughout the skeleton from E14 (49). ATF4, which is highly expressed in all osteoblasts of the skeleton, is necessary to maintain the osteoblast phenotype (49). In addition, ATF4 is a substrate of RSK2 that encodes a growth factor-regulated kinase for the timely onset of osteoblast differentiation, for terminal differentiation of osteoblasts, and for osteoblast-specific gene expression. The activity of RSK2 is controlled by protein stability and phosphorylation (49) (Figure 1).

ATF4-deficient mice, which often die perinatally and are runted, show delayed differentiation of osteoblasts and reduced expression of bone sialoprotein (Bsp) and osteocalcin (49). ATF4 expression is markedly reduced in osteoblasts in Runx2-deficient embryos, demonstrating that its expression in osteoblasts is dependent on Runx2 expression (49). Even in non-osteoblastic cells, the forced expression of ATF4 induces osteoblast-specific gene expression (79). Moreover, the cooperative interactions between ATF4 and Runx2 activate osteocalcin expression, which leads to osteoblast differentiation and bone formation (80). ATF4 is also important to the anabolic action of parathyroid hormone (PTH) on the skeleton (81). In ATF4-deficient osteoblasts, PTH-stimulated proliferation and survival are significantly suppressed and PTH-induced differentiation is also reduced. The PTH-dependent increase in osteoblast differentiation is regulated by interacting with ATF4 in Osx promoter, demonstrating that ATF4 plays an important role in the PTH-mediated induction of Osx as well as the terminal differentiation and function of osteoblasts (81). Furthermore, osteoblast-specific ATF4 overexpression regulates glucose metabolism in the body (82) and ablation of ATF4 in chondrocytes delays endochondral ossification, suggesting that ATF4 may function in chondrocytes as well as osteoblasts (83).

5. SUMMARY

Bone formation by chondrocytes and osteoblasts is regulated by multiple transcription factors. Among them, three major transcriptional regulators, Runx2, Osx, and ATF4, were reviewed in this chapter. Targeted ablation or overexpression of critical transcription factors in bone cells causes the alteration of skeletal phenotypes representing bone diseases. Genetic studies of skeletal diseases are providing important insights into the study of developmental mechanism as well as the roles of individual genes. Correlating these clinical phenotypes in humans with the identified molecular alterations in *in vivo* mouse models provides the possibility of clinical application to diagnostics and treatment for bone diseases in humans. A better understanding of their molecular mechanisms for controlling bone formation would help enable identification of pathogenic causes and development of novel therapeutics for skeletal diseases.

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7. REFERENCES

1. G. Karsenty and E.F. Wagner: Reaching a genetic and molecular understanding of skeletal development. *Dev Cell* 2, 389-406 (2002)

2. G. Karsenty, H.M. Kronenberg and C. Settembre: Genetic control of bone formation. *Annu Rev Cell Dev Biol* 25, 629-648 (2009)

3. C. Hartmann: Transcriptional networks controlling skeletal development. *Curr Opin Genet Dev* 19, 437-443 (2009)

4. G. Karsenty: The complexities of skeletal biology. *Nature* 423, 316-318 (2003)

5. B.R. Olsen, A.M. Reginato and W. Wang: Bone development. *Annu Rev Cell Dev Biol* 16, 191-220 (2000)

6. G.A. Wallis: Bone growth: coordinating chondrocyte differentiation. *Curr Biol* 6, 1577-1580 (1996)

7. J.S. Price, B.O. Oyajobi and R.G. Russell: The cell biology of bone growth. *Eur J Clin Nutr* Suppl 1, S131-S149 (1994)

8. B.R. Olsen: Role of cartilage collagens in formation of the skeleton. *Ann N Y Acad Sci* 785, 124-130 (1996)

9. J.P. Lannotti: Growth plate physiology and pathology. *Orthop Clin North Am* 21, 1-17 (1990)

10. P. Ducy, R. Zhang, V. Geoffroy, A. Ridall and G. Karsenty: Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell* 89, 747-754 (1997)

11. H.M. Kronenberg: Developmental regulation of the growth plate. *Nature* 423, 332-336 (2003)

12. T.H. Vu, J.M. Shipley, G. Bergers, J.E. Berger, J.A. Helms, D. Hanahan, S.D. Shapiro, R.M. Senior and Z. Werb: MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* 93, 411-422 (1998)

13. H. Akiyama: Control of chondrogenesis by the transcription factor Sox9. *Mod Rheumatol* 18, 213-219 (2008)

14. Q. Zhao, H. Eberspaecher, V. Lefebvre and B. de Crombrugghe: Parallel expression of Sox9 and Col2a1 in cells undergoing chondrogenesis. *Dev Dyn* 209, 377-386 (1997)

15. V. Lefebvre, R.R. Behringer and B. de Crombrugghe: L-Sox5, Sox6 and Sox9 control essential steps of the chondrocyte differentiation pathway. *Osteoarthritis Cartilage* 9 Suppl A, S69-S75 (2001)

16. H. Akiyama, M.C. Chaboissier, J.F. Martin, A. Schedl and B. de Crombrugghe: The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. *Genes Dev* 16, 2813-2828 (2002)

17. H. Akiyama, J.P. Lyons, Y. Mori-Akiyama, X. Yang, R. Zhang, Z. Zhang, J.M. Deng, M.M. Taketo, T.

Nakamura, R.R. Behringer, P.D. McCrea and B. de Crombrugghe: Interactions between Sox9 and beta-catenin control chondrocyte differentiation. *Genes Dev* 18, 1072-1087 (2004)

18. L. Koziel, M. Wuelling, S. Schneider and A. Vortkamp: Gli3 acts as a repressor downstream of Ihh in regulating two distinct steps of chondrocyte differentiation. *Development* 132, 5249-5260 (2005)

19. E. Mau, H. Whetstone, C. Yu, S. Hopyan, J.S. Wunder and B.A. Alman: PTHrP regulates growth plate chondrocyte differentiation and proliferation in a Gli3 dependent manner utilizing hedgehog ligand dependent and independent mechanisms. *Dev Biol* 305, 28-39 (2007)

20. B. St-Jacques, M. Hammerschmidt and A.P. McMahon: Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev* 13, 2072-2086 (1999)

21. F. Long, X.M. Zhang, S. Karp, Y. Yang and A.P. McMahon: Genetic manipulation of hedgehog signaling in the endochondral skeleton reveals a direct role in the regulation of chondrocyte proliferation. *Development* 128, 5099-5108 (2001)

22. A. Vortkamp, K. Lee, B. Lanske, G.V. Segre, H.M. Kronenberg and C.J. Tabin: Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. *Science* 273, 613-622 (1996)

23. A.C. Karaplis, A. Luz, J. Glowacki, R.T. Bronson, V.L. Tybulewicz, H.M. Kronenberg and R.C. Mulligan: Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone related peptide gene. *Genes Dev* 8, 277-289 (1994)

24. E.C. Weir, W.M. Philbrick, M. Amling, L.A. Neff, R. Baron and A.E. Broadus: Targeted overexpression of parathyroid hormone-related peptide in chondrocytes causes chondrodysplasia and delayed endochondral bone formation. *Proc Natl Acad Sci USA* 93, 10240-10245 (1996)

25. E. Schipani, B. Lanske, J. Hunzelman, A. Luz, C.S. Kovacs, K. Lee, A. Pirro, H.M. Kronenberg and H. Jüppner: Targeted expression of constitutively active receptors for parathyroid hormone and parathyroid hormone-related peptide delays endochondral bone formation and rescues mice that lack parathyroid hormone related peptide. *Proc Natl Acad Sci USA* 94, 13689-13694 (1997)

26. Y. Maeda, E. Schipani, M.J. Densmore and B. Lanske: Partial rescue of postnatal growth plate abnormalities in Ihh mutants by expression of a constitutively active PTH/PTHrP receptor. *Bone* 46, 472-478 (2010)

27. M. Iwasaki, A.X. Le and J.A. Helms: Expression of indian hedgehog, bone morphogenetic protein 6 and gli during skeletal morphogenesis. *Mech Dev* 69, 197-202 (1997)

28. D.B. Pateder, C.M. Ferguson, A.M. Ionescu, E.M. Schwarz, R.N. Rosier, J.E. Puzas and R.J. O'Keefe: PTHrP expression in chick sternal chondrocytes is regulated by TGF-beta through Smad-mediated signaling. *J Cell Physiol* 188, 343-351 (2001)

29. C.M. Ferguson, E.M. Schwarz, P.R. Reynolds, J.E. Puzas, R.N. Rosier and R.J. O'Keefe: Smad2 and 3 mediate transforming growth factor-beta1-induced inhibition of chondrocyte maturation. *Endocrinology* 141, 4728-4735 (2000)

30. H.A. Pedrozo, Z. Schwartz, R. Gomez, A. Ornoy, W. Xin-Sheng, S.L. Dallas, L.F. Bonewald, D.D. Dean and B.D. Boyan: Growth plate chondocytes store latent transforming growth factor (TGF)-beta 1 in their matrix through latent TGF-beta 1 binding protein-1. *J Cell Physiol* 177, 343-354 (1998)

31. D. Benayahu, U.D. Akavia and I. Shur: Differentiation of bone marrow stroma derived mesenchymal cells. *Curr Med Chem* 14, 173-179 (2007)

32. J.E. Aubin, F. Liu, L. Malaval and A.K. Gupta: Osteoblast and chondroblast differentiation. *Bone* 17, 77S-83S (1995)

33. P. Ducy, T. Schinke and G. Karsenty: The osteoblast: a sophisticated fibroblast under central surveillance. *Science* 289, 1501-1504 (2000)

34. S. Harada and G.A. Rodan: Control of osteoblast function and regulation of bone mass. *Nature* 423, 349-355 (2003)

35. G. Karsenty: Minireview: transcriptional control of osteoblast differentiation. *Endocrinology* 142, 2731-2733 (2001)

36. T. Komori: Regulation of bone development and extracellular matrix protein genes by Runx2. *Cell Tissue Res* 339, 189-195 (2010)

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

38. L.F. Bonewald: Osteocytes as dynamic multifunctional cells. *Ann N Y Acad Sci* 1116, 281-290 (2007)

39. D.A. Glass II and G. Karsenty: Minireview: *In vivo* analysis of Wnt signaling in bone. *Endocrinology* 148, 2630-2634 (2007)

40. S.L. Cheng, J.S. Shao, N. Charlton-Kachigian, A.P. Loewy and D.A. Towler: MSX2 promotes osteogenesis and suppresses adipogenic differentiation of multipotent mesenchymal progenitors. *J Biol Chem* 278, 45969-45977 (2003)

41. F. Ichida, R. Nishimura, K. Hata, T. Matsubara, F. Ikeda, K. Hisada, H. Yatani, X. Cao, T. Komori, A.

Yamaguchi and T. Yoneda: Reciprocal roles of Msx2 in regulation of osteoblast and adipocyte differentiation. *J Biol Chem* 279, 34015-34022 (2004)

42. B. Clarke: Normal bone anatomy and physiology. *Clin J Am Soc Nephrol* Suppl 3, S131-S139 (2008)

43. S.L. Teitelbaum: Bone resorption by osteoclasts. *Science* 289, 1504-1508 (2000)

44. S. Zhang, C. Liu, P. Huang, S. Zhou, J. Ren, Y. Kitamura, P. Tang, Z. Bi and B. Gao: The affinity of human RANK binding to its ligand RANKL. *Arch Biochem Biophys* 487, 49-53 (2009)

45. W.J. Boyle, W.S. Simonet and D.L. Lacey: Osteoclast differentiation and activation. *Nature* 423, 337-342 (2003)

46. L.G. Raisz: Physiology and pathophysiology of bone remodeling. *Clin Chem* 45, 1353-1358 (1999)

47. I. Fernández-Tresguerres-Hernández-Gil, M.A. Alobera-Gracia, M. del-Canto-Pingarrón and L. Blanco-Jerez: Physiological bases of bone regeneration II. The remodeling process. *Med Oral Patol Oral Cir Bucal* 11, E151-E157 (2006)

48. T. Komori: Regulation of bone development and maintenance by Runx2. *Front Biosci* 13, 898-903 (2008)

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

50. R.T. Franceschi, C. Ge, G. Xiao, H. Roca and D. Jiang: Transcriptional regulation of osteoblasts. *Cells Tissues Organs* 189, 144-152 (2009)

51. P. Ducy, V. Geoffroy and G. Karsenty: Study of osteoblast-specific expression of one mouse osteocalcin gene: characterization of the factor binding to OSE2. *Connect Tissue Res* 35, 7-14 (1996)

52. T. Komori, H. Yagi, S. Nomura, A. Yamaguchi, K. Sasaki, K. Deguchi, Y. Shimizu, R.T. Bronson, Y.H. Gao, M. Inada, M. Sato, R. Okamoto, Y. Kitamura, S. Yoshiki and T. Kishimoto: Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 89, 755-764 (1997)

53. M. Inada, T. Yasui, S. Nomura, S. Miyake, K. Deguchi, M. Himeno, M. Sato, H. Yamagiwa, T. Kimura, N. Yasui, T. Ochi, N. Endo, Y. Kitamura, T. Kishimoto and T. Komori: Maturational disturbance of chondrocytes in Cbfa1-deficient mice. *Dev Dyn* 214, 279-290 (1999)

54. C. Ueta, M. Iwamoto, N. Kanatani, C. Yoshida, Y. Liu, M. Enomoto-Iwamoto, T. Ohmori, H. Enomoto, K. Nakata, K. Takada, K. Kurisu and T. Komori: Skeletal malformations caused by overexpression of Cbfa1 or its dominant negative form in chondrocytes. *J Cell Biol* 153, 87-100 (2001)

55. S. Takeda, J.P. Bonnamy, M.J. Owen, P. Ducy and G. Karsenty: Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice. *Genes Dev* 15, 467-481 (2001)

56. W. Liu, S. Toyosawa, T. Furuichi, N. Kanatani, C. Yoshida, Y. Liu, M. Himeno, S. Narai, A. Yamaguchi and T. Komori: Overexpression of Cbfa1 in osteoblasts inhibits osteoblast maturation and causes osteopenia with multiple fractures. *J Cell Biol* 155, 157-166 (2001)

57. F. Otto, A.P. Thornell, T. Crompton, A. Denzel, K.C. Gilmour, I.R. Rosewell, G.W. Stamp, R.S. Beddington, S. Mundlos, B.R. Olsen, P.B. Selby and M.J. Owen: Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell* 89, 765-771 (1997)

58. P. Bialek, B. Kern, X. Yang, M. Schrock, D. Sosic, N. Hong, H. Wu, K. Yu, D.M. Ornitz, E.N. Olson, M.J. Justice and G. Karsenty: A twist code determines the onset of osteoblast differentiation. *Dev Cell* 6, 423-435 (2004)

59. H.M. Kronenberg: Twist genes regulate Runx2 and bone formation. *Dev Cell* 6, 317-318 (2004)

60. M. Oukka, S.T. Kim, G. Lugo, J. Sun, L.C. Wu and L.H. Glimcher: A mammalian homolog of Drosophila schnurri, KRC, regulates TNF receptor-driven responses and interacts with TRAF2. *Mol Cell* 9, 121-131 (2002)

61. D.C. Jones, M.N. Wein, M. Oukka, J.G. Hofstaetter, M.J. Glimcher and L.H. Glimcher: Regulation of adult bone mass by the zinc finger adapter protein Schnurri-3. *Science* 312, 1223-1227 (2006)

62. G. Dobreva, M. Chahrour, M. Dautzenberg, L. Chirivella, B. Kanzler, I. Fariñas, G. Karsenty and R. Grosschedl: SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. *Cell* 125, 971-986 (2006)

63. S. Kim, T. Koga, M. Isobe, B.E. Kern, T. Yokochi, Y.E. Chin, G. Karsenty, T. Taniguchi and H. Takayanagi: Stat1 functions as a cytoplasmic attenuator of Runx2 in the transcriptional program of osteoblast differentiation. *Genes Dev* 17, 1979-1991 (2003)

64. T.M. Schroeder, R.A. Kahler, X. Li and J.J. Westendorf: Histone deacetylase 3 interacts with runx2 to repress the osteocalcin promoter and regulate osteoblast differentiation. *J Biol Chem* 279, 41998-42007 (2004)

65. R.B. Vega, K. Matsuda, J. Oh, A.C. Barbosa, X. Yang, E. Meadows, J. McAnally, C. Pomajzl, J.M. Shelton, J.A. Richardson, G. Karsenty and E.N. Olson: Histone

deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. *Cell* 119, 555-566 (2004)

66. M. Zhao, M. Qiao, S.E. Harris, B.O. Oyajobi, G.R. Mundy and D. Chen: Smurf1 inhibits osteoblast differentiation and bone formation *in vitro* and *in vivo*. *J Biol Chem* 279, 12854-12859 (2004)

67. M. Yamashita, S.X. Ying, G.M. Zhang, C. Li, S.Y. Cheng, C.X. Deng and Y.E. Zhang: Ubiquitin ligase Smurf1 controls osteoblast activity and bone homeostasis by targeting MEKK2 for degradation. *Cell* 121, 101-113 (2005)

68. H. Akiyama, J.E. Kim, K. Nakashima, G. Balmes, N. Iwai, J.M. Deng, Z. Zhang, J.F. Martin, R.R. Behringer, T. Nakamura and B. de Crombrugghe: Osteo-chondroprogenitor cells are derived from Sox9 expressing precursors. *Proc Natl Acad Sci USA* 102, 14665-14670 (2005)

69. W.Y. Baek, M.A. Lee, J.W. Jung, S.Y. Kim, H. Akiyama, B. de Crombrugghe and J.E. Kim: Positive regulation of adult bone formation by osteoblast-specific transcription factor Osterix. *J Bone Miner Res* 24, 1055-1065 (2009)

70. W.Y. Baek, B. de Crombrugghe and J.E. Kim: Postnatally induced inactivation of Osterix in osteoblasts results in the reduction of bone formation and maintenance. *Bone* 46, 920-928 (2010)

71. J.E. Kim, K. Nakashima and B. de Crombrugghe: Transgenic mice expressing a ligand-inducible cre recombinase in osteoblasts and odontoblasts: a new tool to examine physiology and disease of postnatal bone and tooth. *Am J Pathol* 165, 1875-1882 (2004)

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

73. A. Ulsamer, M.J. Ortuño, S. Ruiz, A.R. Susperregui, N. Osses, J.L. Rosa and F. Ventura: BMP-2 induces Osterix expression through up-regulation of Dlx5 and its phosphorylation by p38. *J Biol Chem* 283, 3816-3826 (2008)

74. T. Matsubara, K. Kida, A. Yamaguchi, K. Hata, F. Ichida, H. Meguro, H. Aburatani, R. Nishimura and T. Yoneda: BMP2 regulates Osterix through Msx2 and Runx2 during osteoblast differentiation. *J Biol Chem* 283, 29119-29125 (2008)

75. C. Zhang, K. Cho, Y. Huang, J. P. Lyons, X. Zhou, K. Sinha, P. D. McCrea and B. de Crombrugghe: Inhibition of Wnt signaling by the osteoblast-specific transcription factor Osterix. *Proc Natl Acad Sci USA* 105, 6936-6941 (2008)

76. K.M. Sinha, H. Yasuda, M.M. Coombes, S.Y.R. Dent and B. de Crombrugghe: Regulation of the osteoblast-specific transcription factor Osterix by NO66, a Jumonji family histone demethylase. *EMBO J* 29, 68-79 (2010)

77. K.E. Govoni, G.R. Linares, S.T. Chen, S. Pourteymoor and S. Mohan. T-box 3 negatively regulates osteoblast differentiation by inhibiting expression of osterix and runx2. *J Cell Biochem* 106, 482-490 (2009)

78. L.M. Mielnicki and S.C. Pruitt: Isolation and nucleotide sequence of a murine cDNA homologous to human activating transcription factor 4. *Nucleic Acids Res* 19, 6332 (1991)

79. X. Yang and G. Karsenty: ATF4, the osteoblast accumulation of which is determined post-transcriptionally, can induce osteoblast-specific gene expression in non-osteoblastic cells. *J Biol Chem* 279, 47109-47114 (2004)

80. G. Xiao, D. Jiang, C. Ge, Z. Zhao, Y. Lai, H. Boules, M. Phimphilai, X. Yang, G. Karsenty and R.T. Franceschi: Cooperative interactions between activating transcription factor 4 and Runx2/Cbfa1 stimulate osteoblast-specific osteocalcin gene expression. *J Biol Chem* 280, 30689-30696 (2005)

81. S. Yu, R.T. Franceschi, M. Luo, J. Fan, D. Jiang, H. Cao, T.G. Kwon, Y. Lai, J. Zhang, K. Patrene, K. Hankenson, G.D. Roodman and G. Xiao: Critical role of activating transcription factor 4 in the anabolic actions of parathyroid hormone in bone. *PLoS ONE* 4, e7583 (2009)

82. T. Yoshizawa, E. Hinoi, D.Y. Jung, D. Kajimura, M. Ferron, J. Seo, J.M. Graff, J.K. Kim and G. Karsenty: The transcription factor ATF4 regulates glucose metabolism in mice through its expression in osteoblasts. *J Clin Invest* 119, 2807-2817 (2009)

83. W. Wang, N. Lian, L. Li, H.E. Moss, W. Wang, D.S. Perrien, F. Elefteriou and X. Yang: Atf4 regulates chondrocyte proliferation and differentiation during endochondral ossification by activating Ihh transcription. *Development* 136, 4143-4153 (2009)

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