1

Transcriptional Control of Osteogenesis

Malgorzata Witkowska-Zimny
Department of Biophysics and Human Physiology, Medical University of Warsaw, Poland

1. Introduction

One of the key issues in studying organogenesis is to understand the mechanisms underlying the differentiation of progenitor cells into more specialized cells of individual tissue. The formation and regeneration of each tissue is associated with a cascade of signals involving a sequential activation of successive genes in response to transcriptional regulators, growth factors, cytokines and hormones. Mediators such as fibroblast growth factor (FGF), transforming growth factor beta (TGF-β), insulin-like growth factors (IGF) and many other proteins influence the early stage of cell and tissue formation. The factors are not tissue-specific and control the proliferation and differentiation of most cell types. The proper tissue formation, maturation and function is controlled by factors unique to particular tissue.

Transcriptional regulation is the most important step controlling the decision which genes will be expressed at a given time, thus affect the fate of cells. The recent progress in molecular biology, animal models and development of the skeletal phenotype induced by genetic mutations in humans, led to a better understanding of the role of transcriptional factors that govern bone formation and are specifically activated during osteogenesis.

The development of the vertebrate skeletal elements may take place through two different mechanisms: osteoblastogenesis and osteochondrogenesis, and relies on the differentiation of the required cell types: osteoblasts and chondrocytes (membranous and endochondral/cartilaginous osteogenesis, respectively), which are derived from common mesenchymal stem cells (Fig. 1.).

Osteoblastogenesis (membranous osteogenesis) takes place in the mesenchymal membrane, where osteoblast progenitor cells differentiate directly from embryonic condensed mesenchyme, mature to osteoblasts and begin to secrete type I collagen, proteoglycans and intercellular substance of the organic component of bone – osteoid. These mechanisms are responsible for the development of skull bones, facial bones and parially colarbone.

Most of the vertebrate skeleton develops through endochondral ossification, whereby a cartilaginous template is initially formed and subsequently mineralized and replaced bone. Endochondral bone formation (cartilaginous osteogenesis) takes place in long bones starting with the condensation of skeletal precursors, proceeding to the formation of a cartilagenous template. Chondrocytes at the condensation centre stop proliferating and become hypertrophic (enlarged). This process induces differentiation of osteoblast precursors in the perichondrium adjacent to the region of hypertrophic cells. Perichondrial cells adjacent to hypertrophic chondrocytes become osteoblasts, forming bone collar. At the subsequent steps hypertrophic chondrocytes secrete a mineralized matrix and die through apoptosis.

Vascular invasion follows, bringing osteoblast progenitors from the bone collar into the centre of the future bone. Chondrocytes continue to proliferate, lengthening the bone. Osteoblasts of primary spongiosa are precursors of eventual trabecular bone; osteoblasts of bone collar become cortical bone. At the end of the bone, the secondary ossification centre forms through cycles of chondrocyte hypertrophy, vascular invasion and osteoblast activity. A bone marrow expands in marrow space along with stromal cells.

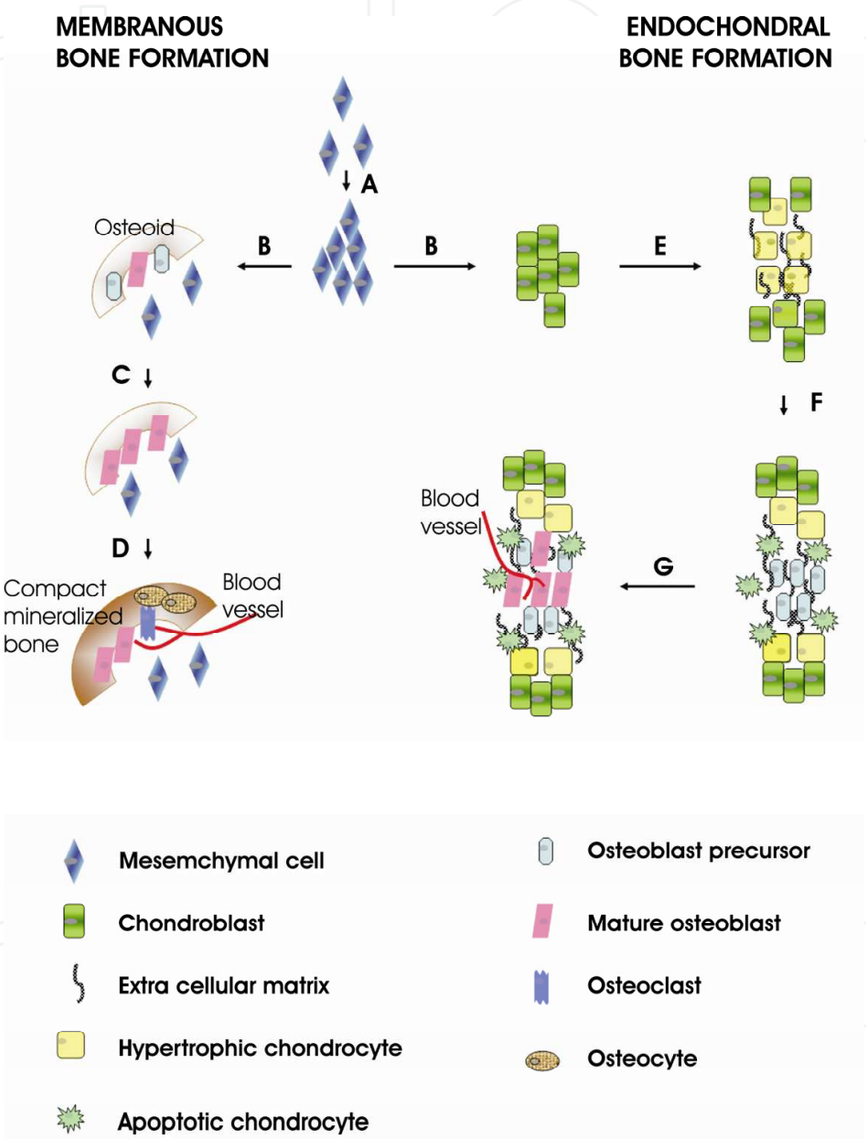


Fig. 1. Schematic representation of membranous and endochondral bone formation. A - mesenchymal cells condensation and proliferation; B – differentiation; C – production of mature osteoblasts; D – osteoblast differente into osteocytes,blood vessels invade and together with osteoblast start to make bone utilize the mineralized matrix; E – synthesis of extracellular matrix and chondrocytes grow to become hypertrophic; F – apoptosis of hypertrophic chondrocytes and osteoblast invasion; G – vascularization of cartilage and osteoblast maturation, secondary ossification center formation are not shown.

All of phenotypic and physiological changes in mesenchymal cells arise as a result of regulatory events at the genetic level. Mutations or deregulation of these processes lead to skeletal malformation, and/or susceptibility to injury. Over the past years on the basis of *in vivo* and *in vitro* studies various transcription factors have been identified that play important roles for skeletal formation being either active in osteoblasts and chondrocytes or in both cell types. The molecular mechanisms controlling the chondrogenic and osteogenic program are becoming elucidated; however, major difficulty in the overall understanding of bone formation is to combine all scattered pieces of information considering the function and importance of individual genes and their products into comprehensive signal network. This chapter presents recent findings on the role of the most important and unique genetic regulators supporting bone formation.

2. Transcription control of osteoblastogenesis

The specific structure of bone, its function and metabolism are a result of the processes of bone formation, resorption, mineral homeostasis and bone regeneration. Osteoblasts are bone-forming cells that synthesize and mineralize extracellular matrix. In membranous bone formation, they arise from multipotential mesenchymal stem cells under the influence of growth factors, hormones and cytokines. Differentiation of progenitor cells into osteoblast *in vitro* has been induced by the presence of osteogenic supplements in cultured medium (dexamethasone, ascorbic acid, vitamin D3, β-glycerophosphate). The proper maturation and osteoblast function is directly related to the expression of two key transcription factors of osteoblastogenesis: Runx2 and Osterix.

2.1 Runx2 (Runt-related transcription factor 2)

The transcriptional control of the proliferation, growth and differentiation of mesenchymal stem cells into mature bone cells is primarily controlled by Runx2 (also known as CBFA1, AML3 or OSF2). Numerous *in vitro* studies have shown that Runx2 is a positive regulator of gene expression, whose products are bone extracellular matrix proteins, such as type I collagen, osteopontin, bone sialoprotein and osteocalcin (Komori, 2006). Runx2 is frequently described as the master regulator of osteoblastogenesis. Its deficiency in homozygots leads to different types of bone dysplasia, consisting of genetically determined disorders in the structure of the skeleton (Marie, 2008). Runx2 is also involved in processes related to the maturation of cartilage cells (Makita et al., 2008). However, during dentinogenesis, Runx2 expression is downregulated, and Runx2 inhibits the terminal differentiation of odontoblast. Runx2 expression has also been recently demonstrated in non-skeletal tissues such as breast, brain, sperm and T cells (Leong et al., 2009). Therefore, it may play a role in transmitting epigenetic information encoded in DNA. The mechanism for regulating the expression of genetic information by Runx2 has been intensively studied for many years. Runx2 is a DNA-binding transcriptional factor that interacts with the promoters of specific target genes through the Runt domain. Its potential binding site has been identified at target promoters, for example in the promoter region of sialoproteins between position -84 to -79 and -184 to -179 relative to the transcription start point (Paz et al., 2005). Regions recognized by Runx2 demonstrate a consensus sequence (PuACCPuCa) described as an osteoblast specific element (OSE2). Runx2 belongs to the Runt family. It should be noted that the other two regulators of the Runt family, Runx1 and Runx3, also participate in the induction of osteoblastic genes. Amino acid sequence alignments of all main Runt family proteins are shown in Fig. 2.

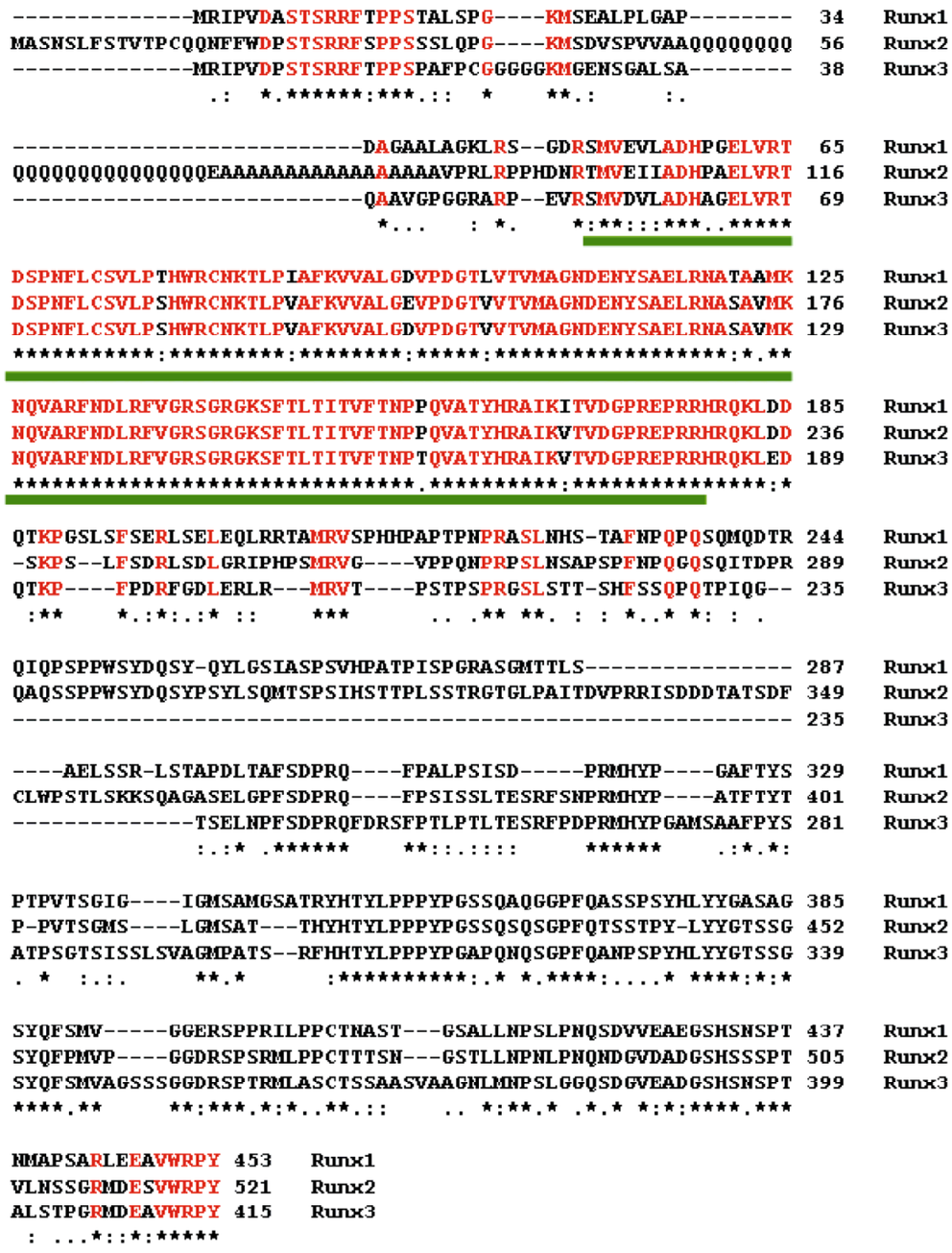


Fig. 2. Amino acid sequence alignments of human Runt family transcriptional regulators: Runx1, Runx2 and Runx3. Residues conserved are colored in red and marked with a star. The dots indicate amino acids with very similar properties. Green bar below the sequence alignment represents DNA binding motif - Runt domain. Sequence accession numbers: Q01196, Q13950, Q13761, respectively.

Two distinct mechanisms involving osteoblast-mediated membranous and endochondrial ossification have been proposed: Runx1 participates in membranous bone formation which corresponds to the involvement of this protein in the early stages of osteoblastogenesis,

while Runx2 plays an exclusive role in both membranous and endochondral bone forming processes that is in the process of osteoblast maturation. Runx3 is engaged in the differentiation of chondrocytes in cartilaginous ossification. The Runt family regulators are proteins that have transcription isoforms. Three isoforms of Runx2 protein are a result of complex process of alternative splicing (Li & Xiao, 2007). However, the specific molecular mechanism involved in the potential isoform functions in bone formation are not well understood. Positive transcription regulators of Runx2 were identified earlier (bone morphogenetic proteins, homeodomain proteins), while the first reports of proteins that inhibit Runx2 gene expression were published in 2009, when the Nieto group showed Snail1 to be a transcriptional repressor at the Runx promoters. Snail1 is a protein involved in the transformation of epithelial cells in the development of embryonic mesenchymal cells (EMT, called the epithelial mesenchymal transition) (De Frutos et al., 2009). Another repressor of Runx2 is Twist - basic helix-loop-helix transcription factor that regulates differentiation of multiple cell types. Twist inhibits osteoblastogenesis by interaction with Runx2-DNA binding domain and in this way prevents its ability to bind DNA (Bialek et al., 2004). Expression of Runx2 is controlled by Foxo1 which can also directly interact with this transcription factors. Teixeira and coworkers suggested that Foxo1 is an early molecular regulator during mesenchymal cell differentiation into osteoblast exerting its effects through regulation and interaction with Runx2 (Teixeira et al., 2010).

Several studies have indicated that Runx2 is a context-dependent transcriptional activator and repressor and may interact with other regulatory proteins, suggesting a complex mechanism of osteoblastogenesis control by this factor. So far coactivators of a Runx2-dependent transcription include p300 and CBP (CREB-binding protein), which function as transcriptional adapters in interactions with other proteins in multiprotein regulatory complexes. Through direct interaction with Runx2, they up-regulate Runx2-dependent transcription. Runx2 corepressors are components of multiprotein complexes that mediate histone deacetylation and condensation of chromatin, such as the TLE (transducin-like enhancer), mSin3A and HDAC3/4/6. Runx2 interacts with many transcription factors, such as Smad1 and Smad5, Twist, Dlx family, Zfp521. The nature of this cooperation is of great interest to many research teams.

The functional activity of Runx2 is particularly sensitive to posttranslational modification of the protein (e.g. phosphorylation, acetylation, methylation). It seems that the preferred interaction of Runx2 with specific cofactors depends on its post-translational modifications (Bae & Lee, 2006). Although Runx2 and its impact on osteoblast differentiation has been widely accepted, our knowledge on the mechanism underlying the process and factors influencing Runx2 expression or activity still require further studies.

2.2 Osterix

Osterix (SP7, OSX) is another transcriptional regulator, essential for differentiation of progenitor cells into osteoblasts, and hence for bone formation. This protein belongs to the Sp/XKLF family of transcriptional factors. The common feature of these regulators is the presence of a DNA binding domain, consisting of Cys2His2 zinc fingers. The OSX protein is comprised of 431 amino acids and contains three zinc finger motifs located in the C-terminal part. It is also possible to distinguish a proline- and serine-rich activation domain between 141 and 210 amino acids. Osterix transcription is positively regulated by Runx2, and in Runx2 null mutants, Osterix expression does not occur (Nakashima et al., 2002). It is an

osteoblast-specific regulator and its activity has not yet been demonstrated in other cell types. The expression of early markers of osteoblast differentiation (e.g. osteopontin, alkaline phosphatase) in the human osteosarcoma cell line MG63 is not dependent on Osterix (Hatta et al., 2006). In contrast, the activation of late genes, such as osteocalcin, is correlated with the presence of this regulator; thus, it appears that Osterix is a factor required to progress the differentiation of preosteoblasts into mature osteoblasts. Despite its involvement in osteoblast differentiation, Osterix regulatory mechanism is not fully understood yet. Osterix activation region is known to interact with many regulatory proteins, including NFAT (nuclear factor of activated T cell) and primary transcription factor – TF-IIB, or Brg1 – chromatin-remodeling factor. Using matrix-assisted laser desorption ionization time-of-flight mass spectrometry, novel Osterix-interacting factors have been identified, such as RNA helicase A (RHA) (Amorim et al., 2007). Immunoprecipitation and Western blot analysis has shown Osterix to directly associate with RNA helicase A. Hence, RNA helicase A may act as a component in Osterix regulation of osteoblast differentiation. Osterix activity is regulated by various post-translational modifications including phosphorylation and glycosylation (Chu & Ferro, 2005). It has been shown that calcineurin, a protein phosphatase, affects the function of Osterix through direct interaction and altering its posttranslational phosphorylation form. The application of calcineurin inhibitor resulted in an increasing level of phosphorylated form of Osterix. Nevertheless, it remains unclear how the phosphorylation of Osterix occurs and modulates its function.

3. Transcriptional control of osteochondrogenesis

In mammals, most skeletal elements are formed through endochondral bone formation, which is characterized by the initial formation of cartilage molds from mesenchymal condensations and their subsequent replacement by bones. Two groups of transcription factors that control the key steps of chondrocyte differentiation have been identified: Sox (sex reversal Y-related high-mobility group box protein**)** and Runt families.

Natural chondrogenesis is initiated by Indian hedgehog signaling and associated with the expression of the major transcription factor Sox9 that controls downstream genes involved in chondrogenesis, and promoting cells to produce cartilage-specific extracellular matrix including collagen type X, collagen type II alpha 1, cartilage oligomeric matrix protein and proteoglycans. In chondrogenesis, Sox9 requires the activity of two other Sox family members: Sox6 and the large isoform of Sox5. Runt family members has also ability to drive chondrocyte differentiation. The stages of chondrocyte differentiation are regulated by a complex series of signaling molecules and transcription factors but also by paracrine factors such as TGF-β, BMPs, FGFs, and Wnts.

3.1 Sox protein and chondrocyte commitment

Sox9 is considered the master protein that control chondrogenic lineage commitment. Role of Sox9 in chondrogenesis was first demonstrated in a human genetic disease campomelic dysplasia. This disease is caused by heterozygous inactivating mutations in and around the Sox9 gene and is characterized by hypoplasia of all endochondral skeletal elements.

Sox9 belongs to a large family of SRY (sex-determination factors) transcriptional regulators which are characterized by a high mobility group DNA-binding domain (HMG-box). This domain preferentially binds CA/TTTGA/TA/T sequence *in vitro* (Han & Lefebvre, 2008).

Sox9 binds to DNA within the minor groove of double helix. Chondrocyte-specific genes (i.e.: collagen type II alpha1-Col2a1, collagen type XI alpha2-Col11a2) contain Sox9-binding sites, mutations in this DNA region abolished DNA binding of Sox9 inactivated these gene *in vivo* and *in vitro*. Sox9 is required during sequential steps of the chondrocyte differentiation pathway. In skeletal development, Sox9 is expressed during the formation of mature cartilage and mesenchymal condensation in endochondrial ossification except hypertrophic chondrocytes. Abnormal expression of Sox9 leads to severe skeletal disorders like skeletal dysmorphology syndrome or campomelic dysplasia. It was shown in experiments in mouse chimeras using Sox9-double null embryonic stem cells that development of cartilages gene is not carried out without active Sox9. On the other hand, forced expression of Sox9 in non-chondrogenic cells leads to the expression of chondrogenic markers, such as collagen type II alpha1 (Col2a1), aggrecan (Acan) and cartilage oligomeric matrix protein (Comp). Interestingly, overexpression of Sox9 in the cartilage causes a decrease in chondrocyte proliferation and a delay in bone development. This decrease in proliferation may result from binding of Sox9 to β-catenin, the essential component of the canonical Wnt signaling pathway. Along with the observation, overactivation or deletion of β-catenin in chondrocytes resulted in severe skeletal dysplasias. The expression and function of Sox9 is not restricted to chondrogenesis, suggesting that this regulator may cooperate with other factors in various/different cellular processes. The activity of Sox9 is regulated by a number of intracellular factors. The function of Sox9 may be modulated by phosphorylation cAMP-dependent protein kinase A by parathyroid hormone-related peptide signaling pathway (PTHrP). It is, therefore, possible that Sox9 mediates part of PTHrP action to regulate hypertrophic differentiation (Zhao et al., 2009).

Other Sox genes necessary for cartilage formation include Sox5 and Sox6. They are expressed downstream of Sox9 from the prechondrocyte stage and later remain coexpressed with Sox9. Sox5 gene is highly similar to Sox6, and the two proteins share 50% homology with Sox9 in the Sox domain but not in remaining domains. The expression of Sox5 and Sox6 requires Sox9. Roles of Sox5 and Sox6 in chondrogenic differentiation were demonstrated in genetically manipulated mice. Single Sox5 or Sox6 knock-out mice mutants are born with relatively small skeletal defects, whereas double Sox5/Sox6 mutants exhibit a lack of cartilage and endochondral bone formation, becouse Sox5, Sox6, with Sox9 form a trio of transcription factors needed and sufficient for chondrogenesis. Sox5 and Sox6 may form homo- and heterodimers with each other, which bind much more efficiently to pairs of HMG-box binding sites than to single binding sites. Sox5 and Sox6 do not contain any domain that allows for transactivation or -repression of genes transcription and may thus act only to facilitate the organization of transcriptional complexes. Yu Han and Veronique Lefebvre data suggest a new model for the chondrogenic action of the Sox trio: "mesenchymal cells and prechondrocytes express chondrocyte markers at low or undetectable levels because Sox9 has a limited ability to bind to the cartilage enhancers of these genes in the absence of Sox5/Sox6. By inducing Sox5 and Sox6 expression in overtly differentiating chondrocytes, Sox9 gives itself the potential to upregulate its own activity as Sox5/Sox6 now binds to distinct sites on the enhancers and thereby secures Sox9 binding to its recognition site" (Han & Lefebvre, 2008). Only combination of all factors: Sox5, Sox6, and Sox9 successfully induce chondrogenic differentiation *in vivo*. Chondrogenic system *in vitro* via the changes in combination of the three Sox protein levels provide a new chondrogenic differentiation model, which may help us to better understand the mechanism of this process, but also adds a powerful tool to cartilage and bone regenerative medicine.

3.2 Role of Runt family members in osteochondrogenesis

The Runt family of DNA-binding transcription factors regulates cell fate determination in a number of tissues and has been shown to play an essential role in the differentiation of osteoblasts. They play a major role at the late stage of chondrocyte differentiation: in hypertrophic and maturating chondrocytes. In addition to osteoblasts, Runx2 is expressed in the lateral mesoderm, mesenchymal condensations, and chondrocytes. Runx3, which is another Runt family transcription factor, is engaged in the terminal differentiation of chondrocytes (Komori, 2005). Chondrocytes divide and produce a characteristic matrix but then stop dividing, change the matrix they synthesize, and become quite large (hypertrophic). Runx2 and, to a lesser extent, Runx3, are the major transcription factors controlling the crucial steps of osteochondrogenesis. Mice missing Runx2 show a defect in chondrocyte maturation, with lack of hypertrophic chondrocytes in many bones, and mice missing both Runx2 and Runx3 completely lack chondrocytes. The activity of Runx2 and Runx3 is modulated through the interactions with other factors. During chondrogenesis, expression of Runx2, is abolished in cells with heterozygous mutations in and around Sox9 (Akiyama et al., 2005). Dlx5 and Dlx6 proteins cooperate with Runx2 in activation of hypertrophic gene expression as they can physically interact with Runx2 and control chondrocyte hypertrophy *in vivo* and *in vitro* (Chin et al., 2007). Karsenty group results suggest that Runx2, in addition to promoting hypertrophy through its expression in chondrocytes, negatively regulates hyperthrophy by acting in the perichondrium thereby providing an additional level of control in order to coordinate chondrocyte maturation and osteoblastogenesis (Hinoi et al., 2006). By using small interfering RNA-mediated knock-down of Bapx1 (also know as Nkx3.2) in cultured chondrocytes an increase in Runx2 expression was shown, what indicate that Bapx1 act as a negative regulator of chondrocyte maturation (Hartman, 2009).

3.3 c-Maf protein

Chondrocyte differentiation in the growth plate is an important process for the longitudinal growth of endochondral bones. Sox9 and Runx2 are the most often-studied transcriptional regulators of the chondrocyte differentiation processes, but the importance of additional factors is also becoming apparent. One such factor is c-Maf. It belongs to a subfamily of the basic ZIP (bZIP) transcription factor superfamily, which act as key regulators of tissue-specific gene expression and terminal differentiation in many tissues. This regulator is low expressed in immature proliferating chondrocytes but high expressed in late hypertrophic and terminal chondrocytes, making it a candidate for controlling terminal differentiation. There is increasing evidence that c-Maf and its splicing variant Lc-Maf play a role in chondrocyte differentiation in a temporal-spatial manner. Various types of abnormalities in endochodrnal ossification or chondrocytic differentiation are caused by abnormalities concerning target genes regulated by c-Maf and Lc-Maf as well as lack of c-Maf itself (as phenotype of mice lacking c-Maf show) (Hong et al., 2011). Although the differential expression patterns of c-Maf and Lc-Maf during chondrogenesis were described, the functional differences between them are still unknown.

c-Maf can form homodimers as well as heterodimers with other transcription factors. c-Maf binding site was identified: two 13- and 14-base pair palindrome sequences, TGCTGACTCAGCA and TGCTGACGTCAGCA (Kataoka et al., 1994).

Studies of the roles of Maf proteins in chondrocyte differentiation and cartilage are under way, as is the identification of genes directly regulated by c-Maf. A causative role for c-Maf will require more hard evidence from direct experiments.

4. Tissue-nonspecific factors for bone formation

During the development of multicellular organisms, cell fate specification is followed by the sorting of different cell types into distinct domains where the different tissues and organs are formed. It has been shown that the formation and differentiation of tissues and organs during embryogenesis is regulated by the activation of a number of factors, which cannot be considered skeletal specific, although they are thought to play a key role in the differentiation and maturation of the osteoblast phenotype, and were observed in tissues that undergo both membranous and endochondral ossification. Alterations in functions of various other non-bone-specific transcription factors have been also demonstrated to affect osteoblastic differentiation and function. Among the many factors essential for organogenesis, and required for skeletal development are: bone morphogenetic proteins (BMPs), Wingless-type (WNT), homeobox genes HOX/HOM, DLX, MSX, ZPA (regulating the activity of tissue polarity, zone polarizing activity), FGF (fibroblast growth factor), Sonic and Indian Hedgehog (Shh and Ihh, respectively) (Witkowska-Zimny et al., 2010).

4.1 Bone morphogenetic proteins

Bone Morphogenetic Proteins (BMPs) belonging to the superfamily of transforming growth factors β (TGF-β) are important regulators involved in the differentiation process of forming tissues and organs during embryogenesis, including growth and differentiation of mesenchymal stem cells into osteogenic cells (Phimphilai et al., 2006). BMPs also play a key role in tissue regeneration in the post-embryonic period. Several proteins belonging to the group of BMPs have been described, of which BMP2, BMP4, BMP7 are acknowledged as osteogenic BMPs since they have been demonstrated to induce osteoblast differentiation in a variety of cell types. BMPs, which function by activating intracellular SMAD proteins and kinase signaling cascades (MAP, ERK PI3-K/AKT) are involved in the expression of multiple target genes (Osyczka et al., 2005). BMPs signals directly correspond to the early embryogenesis proteins containing homeodomain (called homeodomain proteins) involved in the development of the skeleton (HoxA10, Dlx3) (Hassan et al., 2006). Furthermore, the transcription factor of early ostoblastogenesis, Runx2, is induced in response to the presence of BMP2, by a SMAD-dependent signal transduction pathway (Phimphilai et al., 2006). Leong and colleagues demonstrated that palmitoylation was involved in the BMP2-dependent pathway. The inhibition of palmitoylation reduce osteoblast differentiation and mineralization, but had no effect on cell proliferation (Leong et al., 2009). This study was the first one to show that protein palmitoylation plays an important role in osteoblast differentiation and function.

BMPs also play a role in many stages of chondrogenic differentiation, initiating chondroprogenitor cell determination and differentiation of precursors into chondrocytes, and also at the stage of chondrocyte maturation and terminal differentiation (Pizette & Niswander, 2000; Retting et al., 2009). In addition, signalling through the BMP receptors is required for the maintenance of the articular cartilage in postnatal organisms (Rountree et al., 2004). Moreover, BMPs promote cell death and apoptosis of chondrocytes (Zou & Niswander, 1996).

Despite numerous studies, the regulatory pathway dependent on BMP is still not fully understood. Although the molecular mechanisms of signal transduction by BMPs are not known, recombinant human BMP2 and BMP7 have been successfully used in clinical applications as a factor assisting the regeneration of bone tissue (Bessa et al., 2008).

4.2 WNTs

WNTs are secreted glycoproteins involved in the regulation of embryonic development, as well as in the proliferation and differentiation of many tissues, including bone. WNT signal transmission in the cell occurs via various WNT-dependent pathways, which are always activated by binding WNT proteins to the endothelial Frizzled receptor (Fzd) and its coreceptor (Mbalaviele et al., 2005). Activation of a specific pathway depends on the type of WNT ligand and the current conditions within the cell. Gain- or loss-of-function studies in mice have revealed the function of various component of the pathway. To date 19 WNT ligands and 10 different subtypes of Fzd receptors have been detected. The many players in the WNT cascade hamper the precise elucidation of the mechanism by which WNT signaling specificity is achieved. By far the best characterized cascade is the canonical signaling pathway. It has been reported that binding the WNT to the endothelial Fzd receptor and LRP-5/6 protein (lipoprotein-related protein 5 and 6) on the surface of osteoblast progenitor cells, involves the stabilization of the central player in the canonical WNT pathway - β-catenin, and regulation of multiple transcription factors. The level of β-catenin increases in the cytoplasm, which results in its transport to the nucleus and activation ofosteoblast differentiation genes expression. This process is mediated by transcriptional factors, including Runx2 and Osterix. An appropriate level of the canonical Wnt signalling is crucial for chondrogenesis, demonstrated by the abnormal growth plate phenotype in mice harbouring inactivated β-catenin in chondrocytes (Ryu et al., 2002). β-catenin is highly expressed in mesenchymal cells committed to the chondrocytic lineage but down-regulated at the stage of early chondrogenic differentiation, upon up-regulation of Sox9 (Akiyama et al., 2004). Sox9 interacts with β-catenin and enhances its phosphorylation and subsequent degradation. Wnt signalling is again up-regulated during hypertrophy and promotes chondrocyte hypertrophy and endochondral ossification (Hill et al., 2005). WNT/β-catenin signaling is also important for mechanotransduction and fracture healing (Westendorf et al., 2004; Chen et al., 2007).

4.3 HOX – homeobox proteins

HOX protein family, encoded by a subclass of homebox genes, belongs to the regulators controlling the process of embryogenesis in vertebrates. These homeobox transcriptional factors are capable of binding to specific nucleotide sequences on DNA where they either activate or repress genes. The expression of the HOX genes in the developing area is temporally and spatially dynamic. They are critical for proper formation of skeletal tissue. HoxA and HoxD serve in a dose-dependent manner to regulate the size of specific cartilage elements. A surprising finding was that loss of these genes does not interfere the chondrocyte proliferation and differentation, but the growth of the individual elements is not established properly. Therefore, in controlling osteochondrogenesis they act later to regulate longitudinal growth of skeletal elements (Boulet & Capecchi, 2004).

HoxA10 and other homeobox genes responsible *inter alia* for osteoblastogenesis also participate in the regulation of cell proliferation, differentiation and maturation of osteoblasts in the process of modeling and regeneration of bone tissue in adult organism (Zakany et al., 2007). Research conducted in the last two years has shown the dependence of Runx2 gene expression and Runx2-dependent genes (encoding osteocalcin, alkaline phosphatase, bone sialoprotein) on HoxA10. It also showed that HoxA10, both directly and independently of Runx2, regulates the transcription of certain genes during

osteoblastogenesis. Two mechanisms of HoxA10 action have been proposed: as a component of the BMP2 signaling cascade, prior to Runx2 involvement in the induction of genes as a factor osteoblastogenesis, and as a chromatin modifier in the promoter regions of genes specific to bone tissue. Combinatory mechanisms are operative for a regulated transcription of osteoblast genes through the diversification of sequence-specific activators and repressors that contribute to patterns of gene expression and the multistep process of programming involved in bone formation.

4.4 DLX – Distal-less homeodomain proteins

DLX is a family of transcription regulators containing the homeobox domain, which are activated by a BMP2 signal. Dlx3 expression is synchronized with the stages of osteoblast growth and induced by BMP2 (Hassan et al., 2006). An overexpression of Dlx3 in osteoblast progenitor cells changes the expression of the differentiation markers: type I collagen, osteocalcin and alkaline phosphatase. It has been demonstrated that two members of this family, Dlx3 and Dlx5, up-regulate the endogenous expression of Runx2. Like HoxA10, Dlx3 and Dlx5 may participate in ostoblastogenesis through the activation of Runx gene expression, but also directly through other genes, independently of Runx2. It has been demonstrated that Dlx3 and Dlx5 regulate the synthesis of Runx2, but at different stages of the osteoblast differentiation process: Dlx3 in the early stages of osteoblastogenesis, while Dlx5 in mature osteoblasts (Hassan et al., 2009). DLX proteins may bind to Runx2 promoter region, but only after the removal of another homeobox protein, MSX (mesh-less homeodomain), which acts as a repressor. Dlx3 and Dlx5 binding sites next to Runx2 binding site have been identified in the promoter region of alkaline phosphatase and osteocalcin genes. However, it has been shown that the process of bone tissue differentiation occurs in mutants without the Dlx5 gene. This suggests that the Dlx5 protein acts as a regulator of expression in the multiprotein activation complex and not as the main transcription activator of genes involved in the differentiation of the osteogenic lineage (Samee et al., 2008). The specific regulation mechanism of Runt gene expression, alongside with other Runx2-dependent genes with the participation of several classes of homeotic genes, has been suggested in a few works by Jane B. Lian team (Hassan et al., 2006, 2009).

4.5 MSX proteins

Vertebrate Msx are homeobox-containing genes that bear homology to the *Drosophila* muscle segment homeobox gene. The mammalian Msx gene family consists of three members, named Msx1, Msx2, and Msx3. Msx3 is expressed only in the dorsal neural tube, whereas Msx1 and Msx2 are widely expressed in many organs during embryonic development. Msx proteins interact with other homeodomain proteins to regulate transcription. Heterodimers formed between Msx and other homeodomain proteins such as Dlx2, Dlx5, Lhx2 and Pax3 result in mutual functional antagonism *in vitro*. Msx1 and Msx2 are among the critical factors involved in osteoblastogenesis. Null mutation of Msx2 leads to a number of defects in the construction of the skeleton especially in craniofacial region. Loss-of-function and gain-of-function studies show that in mice as in humans Msx1 and Msx2 are required for normal craniofacial morphogenesis (Alappat et al., 2003). They play a role in crucial processes during limb morphogenesis. Mice homozygous for a null mutation in either Msx1 or Msx2 do not display abnormalities in limb development. By contrast, Msx1; Msx2 double mutants exhibit a severe limb phenotype. A number of data have shown that Msx genes are

downstream targets of BMP signaling in the limb. At early stages, Msx1 and Msx2 are expressed in nearly identical patterns that overlap significantly with BMP2, BMP4 and BMP7.

An antagonistic role of Msx2 has been demonstrated in relation to Dlx5 during osteoblast proliferation and differentiation. Dlx5 is activated in the later stages of osteoblastogenesis, which correlate with increasing levels of proteins characteristic of terminally differentiated osteoblasts, such as osteocalcin, while Msx2 adversely affect these processes. On the basis of these studies, it has been suggested that Msx2 stimulates the process of cell proliferation and inhibits cell differentiation. Several models for the Msx2 and Dlx5 relationship have been proposed. In the first model, Runx2-Msx2 forms a complex that deactivates expression of Runx2 and Runx2-dependent genes. With the increasing levels of Dlx5, a Dlx5-Msx2 complex is formed and free Runx2 protein can in consequence activate specific genes. In the second model for Dlx5 and Msx2 interaction, proteins compete for binding to common binding sites in the promoter region of specific genes and they also regulate each other at the transcriptional level. In both cases a balance between the levels of Msx2 and Dlx5 may be critical for osteoprogenitor cell proliferation and differentiation (Lallemand et al., 2005). It is certain that, Msx with other morphogenes and transcription factors build a complex cellular network that control the development and behavior of cells. It would be of great interest to identify the direct target genes of Msx proteins in *vivo* and their associated cellular processes including proliferation, apoptosis, cell adhesion and migration during organogenesis.

4.6 Hedgehog proteins

Hedgehog (Hh) is evolutionarily conserved family in vertebrates, which include Sonic (Shh), Indian (Ihh), and Desert (Dhh) hedgehogs that control numerous aspects of development: cell growth, survival, and differentiation, and pattern almost every aspect of the vertebrate body plan. Dhh expression is largely restricted to gonads. Ihh is specifically expressed in a limited number of tissues, including primitive endoderm, gut and prehypertrophic chondrocytes in the growth plates of bones. Shh is the most broadly expressed mammalian Hh signaling molecule. During early vertebrate embryogenesis, Shh expressed in midline tissues affects skeletal development and most epithelial tissues. The use of a single morphogen for such a wide variety of functions is possible because cellular responses to Hh depend on the type of responding cell, the amount of Hh received, and the time cells are exposed to it (Varjosalo & Taipale, 2008). During skeletogenesis, Shh and Ihh provide positional information and initiate or maintain cellular differentiation programs regulating the formation of cartilage and bone. Malfunction of the Hh signaling network can cause severe skeletal disorders.

4.6.1 Sonic hedgehog

Shh signaling acts to initiate an osteogenic program of mesenchymal cells. The human Shh has three exons that encodes a 462 amino acid polypeptide. The protein is synthesized as a precursor molecule that undergoes cleavage of a signal peptide and then autoproteolytic cleavage. This reaction mediated by cholesterol leads to a 19 kDa N-terminal product (Shh-N) with the signalling domain and a C-terminal product of 25 kDa (Shh-C) possessing the cleavage domain closely associated with cholesterol transferase activity. The Shh is highly conserved among vertebrates. For example, there is 92.4% identity between human and mouse Shh proteins. Increased Hh signaling promotes osteogenesis in various bone-forming

cells *in vitro*. The stimulatory action of Shh on osteogenic differentiation was already reported in few studies, suggesting a close interaction between Shh and BMP-2 or parathyroid hormone-related peptide (Yuasa et al., 2002.) Shh has been shown to play a key role in patterning of the limb, i.e. misregulation of Shh results in severe limb abnormalities. Hence, Shh is required for proper ZPA (regulating the activity of tissue polarity, zone polarizing activity) signaling and anterior/posterior limb formation (Hill, 2007; Towers et al., 2008, James et al., 2010).

4.6.2 Indian hedgehog

Growth and differentiation of the endochondral skeleton relies on a complex interplay among different signaling factors to regulate the orderly morphogenesis of the skeleton. Among these, Ihh appears to play a central role in coordinating chondrocyte proliferation, chondrocyte differentiation and osteoblast differentiation. During endochondral bone development, Ihh is synthesized by chondrocytes leaving the proliferative pool (prehypertrophic chondrocytes) and by early hypertrophic chondrocytes. Ihh is a master regulator of both chondrocyte and osteoblast differentiation during endochondral bone formation.

Ihh mutants show: (i) reduced chondrocyte proliferation; (ii) initially delayed, then abnormal chondrocyte maturation, and (iii) absence of mature osteoblasts. St-Jacques and coworkers suggest a model in which Ihh coordinates diverse aspects of skeletal morphogenesis through parathyroid hormone-related peptide dependent and independent processes (St-Jacques et al., 1999). Mouse Ihh cDNA encodes a 411 amino acids polypeptide with a predicted 27 amino acids signal peptide. After the post-translational modification arises a 19 kDa lipid-modified protein. At the cell surface, Ihh activity is mediated by binding to the transmembrane receptor, and signaling through the transmembrane G-protein coupled receptor. Hedgehogs, including Ihh are important signaling molecules during embryonic development and are highly conserved within and across species. Mouse and human Ihh share 100% amino acid identity of the signaling domain.

4.7 Zinc finger protein: PLZF and Zfp521

Zinc finger proteins are believed to be one of the most common classes of proteins in humans (approx. 3-4% of human genes encode proteins containing zinc finger domains). One of previously described osteoblastogenesis factor – Osterix also belong to this protein type.

Promyelocytic leukaemia zinc finger protein (Zinc finger protein 145, PLZF) belongs to the family of Krüppel-like zinc finger proteins. It is a transcriptional repressor involved in cell cycle control and has been implicated in limb development, differentiation of myeloid cells, and spermatogenesis. So far little is known about the regulation of PLZF expression.

PLZF is one of the highly expressed genes during *in vitro* osteoblastic differentiation in many human cell types. Small interfering RNA-mediated gene silencing of PLZF results in a reduction of osteoblast-specific genes expression such as alkaline phosphatase, collagen type 1, osteocalcin and even Runx2 genes. These findings indicate that PLZF plays important roles in early osteoblastic differentiation as an upstream regulator of Runx2. Because the expression of PLZF was unaffected by the addition of bone morphogenetic protein 2 *in vitro*, it may indicate that PLZF acts independently of the BMP signaling pathway (Ikeda et al., 2005). The molecular pathways by which PLZF exerts its function in bone formation are still under investigation.

Another regulator from Kruppel-like zinc finger protein family associated with osteogenesis is Zfp521. Zfp521 is expressed in osteoblast precursors, osteoblasts and osteocytes, as well as chondrocytes. Forced expression of Zfp521 in osteoblasts *in vivo* increases bone formation and bone mass. In contrast, overexpression of Zfp521 *in vitro* antagonizes, while knockdown favors, osteoblast differentiation and nodule formation. Zfp521 binds to Runx2, repressing its transcriptional activity (Wu et al., 2009). The balance between Zfp521 and Runx2 may therefore contribute to the regulation of osteoblast differentiation and bone formation.

5. MicroRNAs in skeletal development

One of the first steps in understanding of cell determination requires ascertaining which particular genes are activated in a particular cell, either temporarily or continuously. Microarrays technology are used as a tool for quantitative expression analysis of many gene transcripts in parallel as well as study expression of small non-coding microRNAs that repress mRNA translation and thereby regulate differentiation and development. Post-transcriptional regulation by non-coding RNA molecules has been discovered to be an important mechanism to control cellular differentiation, also during bone formation. An increasing number of miRNAs have been identified to regulate osteoblast differentiation. They promote bone formation by targeting negative regulators of osteogenesis or negatively regulate osteoblastogenesis by targeting important osteogenic factors.

Among many miRNA negatively regulating osteogenesis are Runx2-targeting miRNAs: miR-23a, miR-30c, miR-34c, miR-133a, miR-135a, miR-137, miR-204, miR-205, miR-217, and miR-338 (Zhang et al., 2011a, 2011b). They significantly impede osteoblast differentiation, and their effects can be reversed by the corresponding anti-miRNAs.

Only a few miRNAs have been identified to specifically regulate chondrogenesis and cartilage homeostasis: miR-140 and miR-199 as negative regulators and miR-675 as a positive regulator (Miyaki *et al.*, 2009; 2010;). miR-675, whose expression is upregulated by Sox9, positively regulates chondrocyte specific gene i.e. Col2a1, and in this way promotes chondrogenesis (Dudek *et al.*, 2010; Lin *et al.*, 2009).

6. Conclusion

Bone is a highly dynamic tissue, which is regulated by tissue-specific transcription factors, as well as by the number of homeotic genes, active both during the organization of tissue and organs in the embryonic period as well as in mature bone. The most important factors for osteogenesis are compiled and briefly overview in the Table 1.

Transcriptional regulators control the expression of target genes by the interaction with cofactors, coactivators, chromatin remodelling complexes and finally with the general transcriptional machinery. Their participation in the regulation of bone formation process is complex and require further experimental work to provide understanding of their role and elucidate interactions with other factors of signal cascades. Cellular balance between various regulatory proteins is extremely important. Many studies have been conducted using murine or human cell lines, which are often tumor cell lines e.g. MG63 – human osteosarcoma line. The regulatory pathways and routes of signal transduction in these experimental systems may not correspond to those occurring in healthy human bone cells. Therefore, it is important to enhance our knowledge about proliferation, differentiation and regeneration of bone based on *in vitro* and *in vivo* studies of normal human cells in the

context of natural tissue. From a biomedical point of view, identifying an important regulators of bone formation and regeneration in humans has raised the possibility that manipulating its expression, function, or signalling pathway could have a major therapeutic impact in identifying new targets and opening new avenues for the treatment of bone diseases, such as osteoporosis.

Protein (Synonym) **Full recommended name**	**Length (aa)**	**DNA binding motif (position)**	**Accession number UniProtKB/ Swiss-Prot**	**Gene locus**	**Number of isoforms**
Runx1 (AML1, CBFA2) **Runt-related transcription factor 1**	453	Runt domain (50-178 aa)	Q01196	21q22.3	11
Runx2 (AML3, CBFA1, OSF2, PEBP2A) **Runt-related transcription factor 2**	521	Runt domain (101-229 aa)	Q13950	6p21	3
Runx3 (AML2, CBFA3, PEBP2A3) **Runt-related transcription factor 3**	415	Runt domain (54-182 aa)	Q13761	1p36	2
Osterix (SP7, OSX) **Transcription factor Sp7**	431	Three C2H2-type zinc fingers (294-318 aa; 324-348 aa; 354 – 376 aa)	Q8TDD2	12q13.13	1
Sox9 (SRY-box9) **Transcription factor SOX-9**	509	HMG box DNA-binding domain (105-173 aa)	P48436	17q23	1
Sox5 Transcription factor SOX-5	763	HMG box DNA-binding domain. (556-624 aa)	P35711	12p12.1	4
Sox6 Transcription factor SOX-6	828	HMG box DNA-binding domain (621-689 aa)	P35712	11p15.3	4
c-Maf Transcription factor Maf	373	Leucine-zipper (316-337 aa)	O75444	16q22-q23	2

Table 1. Summary of human main bone tissue-specific transcriptional regulators.

7. Acknowledgment

Studies in the laboratory of the author are supported by grants no. N N302157037 from the Polish funds for scientific research in 2009-2012.

8. References

Akiyama, H., Lyons, J. P., Mori-Akiyama, Y., Yang, X., Zhang, R., Zhang, Z., Deng, J. M., Taketo, M. M., Nakamura, T., Behringer, R. R., McCrea P. D. & de Crombrugghe, B. (2004) Interactions between Sox9 and beta-catenin control chondrocyte differentiation. *Genes Dev,* Vol.18**,** No.9 (May 2004), pp. 1072-1087

Akiyama, H., Kim, J. E., Nakashima, K., Balmes, G., Iwai, N., Deng, J. M., Zhang, Z.; Martin, J. F., Behringer, R. R., Nakamura, T. & de Crombrugghe B. (2005) Osteo-chondroprogenitor cells are derived from Sox9 expressing precursors. *Proc Natl Acad Sci U S A,* Vol.102**,** No.41, (October 2005) pp. 14665-14670

Alappat, S., Zhang, Z. Y. & Chen, Y. P. (2003) Msx homeobox gene family and craniofacial development. *Cell Res,* Vol.13**,** No.6, (December 2003), pp. 429-442

Amorim, B. R., Okamura, H., Yoshida, K., Qiu, L., Morimoto H. & T. Haneji, T. (2007) The transcriptional factor Osterix directly interacts with RNA helicase A. *Biochem Biophys Res Commun,* Vol.355**,** No.2, (April 2007), pp. 347-351

Bae, S. C. & Lee, Y. H. (2006) Phosphorylation, acetylation and ubiquitination: the molecular basis of RUNX regulation. *Gene,* Vol.366**,** No.1, (January 2006), pp. 58-66

Bessa, P. C., Casal, M. & Reis, R. L. (2008) Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). *J Tissue Eng Regen Med,* Vol.2**,** No.2, (March-April 2008), pp. 81-96

Bialek, P., Kern, B., Yang, X., Schrock, M., Sosic, D., Hong, N., Wu, H., Yu, K., Ornitz, D. M., Olson, E. N., Justice, M. J. & Karsenty, G. (2004) A twist code determines the onset of osteoblast differentiation. *Dev Cell,* Vol.6**,** No.3, (March 2004) pp. 423-435

Boulet, A. M. & Capecchi, M. R. (2004) Multiple roles of Hoxa11 and Hoxd11 in the formation of the mammalian forelimb zeugopod. *Development,* Vol.131**,** No.2, (January 2004), pp. 299-309

Chen, Y., Whetstone, H. C., Lin, A. C., Nadesan, P., Wei, Q., Poon, R. & Alman, B. A. (2007) Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. *PLoS Med,* Vol.4, No.7, (July 2007)**,** pp. e249

Chin, H. J., Fisher, M. C., Li, Y., Ferrari, D., Wang, C. K., Lichtler, A. C., Dealy, C. N. & Kosher, R. A. (2007) Studies on the role of Dlx5 in regulation of chondrocyte differentiation during endochondral ossification in the developing mouse limb. *Dev Growth Differ,* Vol.49**,** No. 6, (August 2007), pp. 515-521

Chu, S. & Ferro, T. J. (2005) Sp1: regulation of gene expression by phosphorylation. *Gene,* Vol.348**,** (March 2005) pp. 1-11

de Frutos, C. A., Dacquin, R., Vega, S., Jurdic, P., Machuca-Gayet, I. & Nieto, M. A. (2009) Snail1 controls bone mass by regulating Runx2 and VDR expression during osteoblast differentiation. *EMBO J,* Vol.28**,** No.6, (March 2009), pp. 686-696

Dudek, K. A., Lafont, J. E., Martinez-Sanchez, A. & Murphy, C. L. (2010) Type II collagen expression is regulated by tissue-specific miR-675 in human articular chondrocytes. *J Biol Chem,* Vol.285**,** No.32, (August 2010), pp. 24381-24387

Han, Y. & Lefebvre, V. (2008) L-Sox5 and Sox6 drive expression of the aggrecan gene in cartilage by securing binding of Sox9 to a far-upstream enhancer. *Mol Cell Biol,* Vol.28**,** No. 16, (August 2008), pp. 4999-5013

Hartmann, C. (2009) Transcriptional networks controlling skeletal development. *Curr Opin Genet Dev,* Vol.19, No.5, (October 2009), pp. 437-443

Hassan, M. Q., Tare, R. S., Lee, S. H., Mandeville, M., Morasso, M. I., Javed, A., van Wijnen, J., Stein, J. L., Stein, G. S. & Lian, J. B. (2006) BMP2 commitment to the osteogenic lineage involves activation of Runx2 by DLX3 and a homeodomain transcriptional network. *J Biol Chem,* Vol.281**,** No. 52, (December 2006), pp. 40515-40526

Hassan, M. Q., Saini, S., Gordon, J. A., van Wijnen, A. J., Montecino, M., Stein, J. L., Stein, G. S. & Lian, J. B. (2009) Molecular switches involving homeodomain proteins, HOXA10 and RUNX2 regulate osteoblastogenesis. *Cells Tissues Organs,* Vol.189**,** No. 1-4, pp. 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,* Vol.17**,** No.3, (March 2006), pp. 425-430

Hill, R. E. (2007) How to make a zone of polarizing activity: insights into limb development via the abnormality preaxial polydactyly. *Dev Growth Differ,* Vol.49**,** No.6, (August 2007), pp. 439-448

Hill, T. P., Spater, D., Taketo, M. M., Birchmeier, W. & Hartmann, C. (2005) Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. *Dev Cell,* Vol.8**,** No.5, (May 2005), pp. 727-738

Hinoi, E., Bialek, P., Chen, Y. T., Rached, M. T., Groner, Y., Behringer, R. R., Ornitz, D. M. & Karsenty, G. (2006) Runx2 inhibits chondrocyte proliferation and hypertrophy through its expression in the perichondrium. *Genes Dev,* Vol.20, No.21, (November 2006), pp. 2937-2942

Hong, E., Di Cesare, P. & Haudenschild, D. E (2011) Role of c-Maf in Chondrocyte Differentiation. *Cartilage* Vol. 2 No.1 (January 2011), pp. 27-35

Ikeda, R., Yoshida, K., Tsukahara, S., Sakamoto, Y., Tanaka, H., Furukawa, K. & Inoue, I. (2005) The promyelotic leukemia zinc finger promotes osteoblastic differentiation of human mesenchymal stem cells as an upstream regulator of CBFA1. *J Biol Chem,* Vol.280**,** No.9, (March 2005), pp. 8523

James, A. W., Leucht, P., Levi, B., Carre, A. L., Xu, Y., Helms, J. A. & Longaker, M. T. (2010) Sonic Hedgehog influences the balance of osteogenesis and adipogenesis in mouse adipose-derived stromal cells. *Tissue Eng Part A,* Vol.16**,** No.8, (August 2010), pp. 2605-2616

Kataoka, K., Noda, M. & Nishizawa, M. (1994) Maf nuclear oncoprotein recognizes sequences related to an AP-1 site and forms heterodimers with both Fos and Jun. *Mol Cell Biol,* Vol.14**,** No. 1, (January 1994), pp. 700-712

Komori, T. (2005) Regulation of skeletal development by the Runx family of transcription factors. *J Cell Biochem,* Vol.95**,** No.3, (June 2005), pp. 445-453

Lallemand, Y., Nicola, M. A., Ramos, C., Bach, A., Clement, C. S. & Robert, B. (2005) Analysis of Msx1; Msx2 double mutants reveals multiple roles for Msx genes in limb development. *Development,* Vol.132**,** No.13, (July 2005), pp. 3003-3014

Leong, W. F., Zhou, T., Lim, G. L. & Li, B. (2009) Protein palmitoylation regulates osteoblast differentiation through BMP-induced osterix expression. *PLoS ONE,* Vol.4**,** No.1, (January 2009), e4135

Li, Y. L. & Xiao, Z. S. (2007) Advances in Runx2 regulation and its isoforms. *Med Hypotheses,* Vol.68**,** No.1, (January 2007), pp. 169-175

Lin, E. A., Kong, L., Bai, X. H., Luan, Y. & Liu, C. J. (2009) miR-199a, a bone morphogenic protein 2-responsive MicroRNA, regulates chondrogenesis via direct targeting to Smad1. *J Biol Chem,* Vol.284**,** No. 17, (April 2009), pp. 11326-11335

Makita, N., Suzuki, M., Asami, S., Takahata, R., Kohzaki, D., Kobayashi, S., Hakamazuka, T. & Hozumi, N. (2008) Two of four alternatively spliced isoforms of RUNX2 control osteocalcin gene expression in human osteoblast cells. *Gene,* Vol.413**,** No.1-2, (April 2008), pp. 8-17

Marie, P. J. (2008) Transcription factors controlling osteoblastogenesis. *Arch Biochem Biophys,* Vol.473**,** No.2, (May 2008), pp. 98-105

Mbalaviele, G., Sheikh, S., Stains, J. P., Salazar, V. S., Cheng, S. L., Chen, D. & Civitelli, R. (2005) Beta-catenin and BMP-2 synergize to promote osteoblast differentiation and new bone formation. *J Cell Biochem,* Vol.94**,** No.2, (February 2005), pp. 403-418

Miyaki, S., Nakasa, T., Otsuki, S., Grogan, S. P., Higashiyama, R., Inoue, A., Kato, Y., Sato, T., Lotz, M. K. & Asahara, H. (2009) MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. *Arthritis Rheum,* Vol.60**,** No.9, (September 2009), pp. 2723-2730

Miyaki, S., Sato, T., Inoue, A., Otsuki, S., Ito, Y., Yokoyama, S., Kato, Y., Takemoto, F., Nakasa, T., Yamashita, S., Takada, S., Lotz, M. K., Ueno-Kudo, H. & Asahara, H. (2010) MicroRNA-140 plays dual roles in both cartilage development and homeostasis. *Genes Dev,* Vol.24**,** No.11, (June 2010), pp. 1173-1185

Nakashima, K., Zhou, X., Kunkel, G., Zhang, Z., Deng, J. M., Behringer, R. R. & de Crombrugghe, B. (2002) The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell,* Vol.108**,** No.1, (January 2002), pp. 17-29

Osyczka, A. M. & Leboy, P. S. (2005) Bone morphogenetic protein regulation of early osteoblast genes in human marrow stromal cells is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase signaling. *Endocrinology,* Vol.146**,** No. 8, (August 2005), pp. 3428-3437

Paz, J., Wade, K., Kiyoshima, T., Sodek, J., Tang, J., Tu, Q., Yamauchi, M. & Chen, J. (2005) Tissue- and bone cell-specific expression of bone sialoprotein is directed by a 9.0 kb promoter in transgenic mice. *Matrix Biol,* Vol.24**,** No.5, (August 2005), pp. 341-352

Phimphilai, M., Zhao, Z., Boules, H., Roca, H. & Franceschi, R. T. (2006) BMP signaling is required for RUNX2-dependent induction of the osteoblast phenotype. *J Bone Miner Res,* Vol.21**,** No.4, (April 2006), pp. 637-646

Pizette, S. & Niswander, L. (2000) BMPs are required at two steps of limb chondrogenesis: formation of prechondrogenic condensations and their differentiation into chondrocytes. *Dev Biol,* Vol.219**,** No.2, (March 2000), pp. 237-249

Retting, K. N., Song, B., Yoon, B. S. & Lyons, K. M. (2009) BMP canonical Smad signaling through Smad1 and Smad5 is required for endochondral bone formation. *Development,* Vol.136**,** No.7, (April 2009), pp. 1093-1104

Rountree, R. B., Schoor, M., Chen, H., Marks, M. E., Harley, V., Mishina, Y. & Kingsley, D. M. (2004) BMP receptor signaling is required for postnatal maintenance of articular cartilage. *PLoS Biol,* Vol.2**,** No.11, (November 2004), e355.

Ryu, J. H., Kim, S. J., Kim, S. H., Oh, C. D., Hwang, S. G., Chun, C. H., Oh, S. H., Seong, J. K., Huh, T. L. & Chun, J. S. (2002) Regulation of the chondrocyte phenotype by beta-catenin. *Development,* Vol.129**,** No.23, (December 2002), pp. 5541-5550

Samee, N., Geoffroy, V., Marty, C., Schiltz, C., Vieux-Rochas, M., Levi, G. & de Vernejoul, M. C. (2008) Dlx5, a positive regulator of osteoblastogenesis, is essential for osteoblast-osteoclast coupling. *Am J Pathol,* Vol.173**,** No. 3 (September 2008), pp. 773-780

St-Jacques, B., Hammerschmidt, M. & McMahon, A. P. (1999) Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev,* Vol.13**,** No. 16, (August 1999), pp. 2072-2086

Teixeira, C. C., Liu, Y., Thant, L. M., Pang, J., Palmer, G. & Alikhani, M. (2010) Foxo1, a novel regulator of osteoblast differentiation and skeletogenesis. *J Biol Chem,* Vol.285**,** No. 40, (October 2010), pp. 31055-31065

Towers, M., Mahood, R., Yin, Y. & Tickle, C. (2008) Integration of growth and specification in chick wing digit-patterning. *Nature,* Vol.452**,** No. 7189, (April 2008), pp. 882-886

Varjosalo, M. & Taipale, J. (2008) Hedgehog: functions and mechanisms. *Genes Dev,* Vol.22**,** No.18, (September 2008), pp. 2454-2472

Westendorf, J. J., Kahler, R. A. & Schroeder, T. M. (2004) Wnt signaling in osteoblasts and bone diseases. *Gene,* Vol.341**,** (October 2004), pp. 19-39

Witkowska-Zimny, M., Wrobel, E. & Przybylski, J. (2010) The most importan trancsroptional factors of osteoblastogenesis. *Advances in cell biology*, Vol.2, No.1, (January 2010), pp. 17-28

Wu, M., Hesse, E., Morvan, F., Zhang, J. P., Correa, D., Rowe, G. C., Kiviranta, R., Neff, L., Philbrick, W. M., Horne, W. C. & Baron, R. (2009) Zfp521 antagonizes Runx2, delays osteoblast differentiation in vitro, and promotes bone formation in vivo. *Bone,* Vol.44**,** No.4, (April 2009), pp. 528-536

Yuasa, T., Kataoka, H., Kinto, N., Iwamoto, M., Enomoto-Iwamoto, M., Iemura, S., Ueno, N., Shibata, Y., Kurosawa, H. & Yamaguchi, A. (2002) Sonic hedgehog is involved in osteoblast differentiation by cooperating with BMP-2. *J Cell Physiol,* Vol.193**,** No.2, (November 2002), pp. 225-232

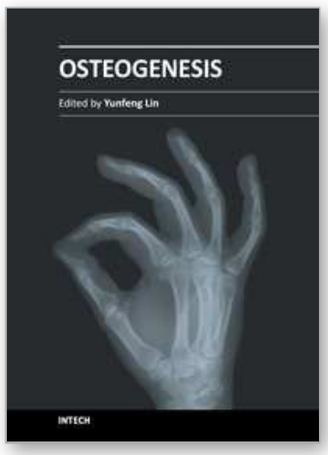
Zakany, J. & Duboule, D. (2007) The role of Hox genes during vertebrate limb development. *Curr Opin Genet Dev,* Vol.17**,** No.4, (August 2007), pp. 359-366

Zhang, Y., Xie, R. L., Croce, C. M., Stein, J. L., Lian, J. B., van Wijnen, A. J. & Stein, G. S. (2011a) A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. *Proc Natl Acad Sci U S A,* Vol.108**,** No.24, (June 2011), pp. 9863-9868

Zhang, J. F., Fu, W. M., He, M. L., Xie, W. D., Lv, Q., Wan, G., Li, G., Wang, H., Lu, G., Hu, X., Jiang, S., Li, J. N., Lin, M. C., Zhang, Y. O. & Kung, H. (2011b) MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. *RNA Biol,* Vol.8, No. 5, (September 2011), pp. 829-838

Zhao, L., Li, G. & Zhou, G. Q. (2009) SOX9 directly binds CREB as a novel synergism with the PKA pathway in BMP-2-induced osteochondrogenic differentiation. *J Bone Miner Res,* Vol.24**,** No.5, (May 2009), pp. 826-836

Zou, H. & Niswander, L. (1996) Requirement for BMP signaling in interdigital apoptosis and scale formation. *Science,* Vol.272**,** No. 5263 (May 1996), pp. 738-741



Osteogenesis

Edited by Prof. Yunfeng Lin

ISBN 978-953-51-0030-0
Hard cover, 296 pages
Publisher InTech
Published online 10, February, 2012
Published in print edition February, 2012

This book provides an in-depth overview of current knowledge about Osteogenesis, including molecular mechanisms, transcriptional regulators, scaffolds, cell biology, mechanical stimuli, vascularization and osteogenesis related diseases. Hopefully, the publication of this book will help researchers in this field to decide where to focus their future efforts, and provide an overview for surgeons and clinicians who wish to be directed in the developments related to this fascinating subject.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Malgorzata Witkowska-Zimny (2012). Transcriptional Control of Osteogenesis, Osteogenesis, Prof. Yunfeng Lin (Ed.), ISBN: 978-953-51-0030-0, InTech, Available from: http://www.intechopen.com/books/osteogenesis/transcriptional-control-of-osteogenesis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen