PROSPECT
Journal of Cellular Biochemistry 112:750–755 (2011)

Journal of Cellular Biochemistry

Signaling Networks in RUNX2-Dependent Bone Development

Toshihisa Komori*

Department of Cell Biology, Unit of Basic Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8588, Japan

ABSTRACT

RUNX2 is an essential transcription factor for osteoblast differentiation and chondrocyte maturation. SP7, another transcription factor, is required for osteoblast differentiation. Major signaling pathways, including FGF, Wnt, and IHH, also play important roles in skeletal development. RUNX2 regulates *Sp7* expression at an early stage of osteoblast differentiation. FGF2 upregulates *Runx2* expression and activates RUNX2, and gain-of-function mutations of FGFRs cause craniosynostosis and limb defect with upregulation of *Runx2* expression. Wnt signaling upregulates *Runx2* expression and activates RUNX2, and RUNX2 induces *Tcf7* expression. IHH is required for *Runx2* expression in osteoprogenitor cells during endochondral bone development, and RUNX2 directly regulates *Ihh* expression in chondrocytes. Thus, RUNX2 regulates osteoblast differentiation and chondrocyte maturation through the network with SP7 and with FGF, Wnt, and IHH signaling pathways during skeletal development. J. Cell. Biochem. 112: 750–755, 2011. © 2010 Wiley-Liss, Inc.

KEY WORDS: RUNX2; FGF; WNT; SP7; IHH, OSTEOBLAST; CHONDROCYTE

RUNX2 is an essential transcription factor for osteoblast and chondrocyte differentiation [Komori, 2005]. RUNX2 belongs to the Runx family, which consists of RUNX1, RUNX2, and RUNX3. These transcription factors form heterodimers with CBFB and bind the consensus sequence, TGPyGGPyPy. These transcription factors act as master regulators in different cell lineages with some overlapping functions. RUNX1 is essential for hematopoietic stem cell differentiation and is involved in acute myeloid leukemia. RUNX3 plays important roles in the growth regulation of gastric epithelial cells and in neurogenesis, and is related to gastric cancer. RUNX1 and RUNX3 are required for thymocyte development, and RUNX3 has a redundant function with RUNX2 in chondrocyte differentiation in a late stage. In this review, the focus is on the interaction of RUNX2 with major signaling pathways, including FGF, Wnt, and IHH, and on SP7, another essential transcription factor for osteoblast differentiation, during bone development.

SP7 AND RUNX2

SP7 is a zinc finger-containing protein which belongs to the Sp/KLF (Kruppel like factor) family of transcription factors [Nakashima et al., 2002]. *Sp7*-deficient ($Sp7^{-/-}$) mice are unable to form bone due to the arrest of osteoblast differentiation. Their mesenchymal cells condense in the perichondrial region, where bone normally forms, and express genes for chondrocyte markers but not bone matrix proteins. Therefore, SP7 is essential for osteoblast differentiation, and $Runx2^{+}Sp7^{-}$ mesenchymal cells (preosteoblasts) are able to differentiate into osteoblasts or chondrocytes [Nakashima et al., 2002]. As *Runx2* is expressed in mesenchymal cells in $Sp7^{-/-}$ mice, SP7 is a downstream molecule of RUNX2. BMP and IGF mediate *Sp7* expression via MAPK and protein kinase D pathways and RUNX2 [Celil and Campbell, 2005]. Further, BMP induces *Sp7* expression in a manner dependent on RUNX2 and independent of RUNX2 through MSX2 [Matsubara et al., 2008] (Fig. 1).

In contrast to RUNX2, the induction of osteoblast differentiation by SP7 is dependent on the cells and culture conditions in vitro [Kim et al., 2006; Maehata et al., 2006; Tu et al., 2006; Fu et al., 2007; Kurata et al., 2007; Kaback et al., 2008; Matsubara et al., 2008; Kärner et al., 2009; Tominaga et al., 2009]. Thus, additional factors are required for SP7-dependent osteoblast differentiation. Although osteoblast-specific deletion of *Sp7* using the 2.3 kb *Col1a1* promoter Cre transgenic mice resulted in osteopenia due to the inhibition of osteoblast differentiation in adult mice [Baek et al., 2009], the important role of SP7 should be in the early stage of osteoblast differentiation. In addition to capacity for the induction of osteoblast differentiation, RUNX2 is involved in vascular invasion

*Correspondence to: Prof. Toshihisa Komori, PhD, Department of Cell Biology, Unit of Basic Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan.
E-mail: komorit@nagasaki-u.ac.jp

Received 1 December 2010; Accepted 2 December 2010 • DOI 10.1002/jcb.22994 • © 2010 Wiley-Liss, Inc.
Published online 28 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

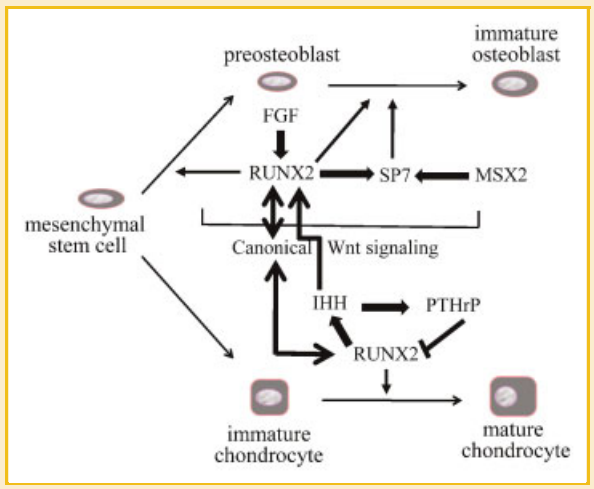

Fig. 1. RUNX2 plays important roles in osteoblast differentiation and chondrocyte maturation. RUNX2, SP7, and canonical Wnt signaling are essential for osteoblast differentiation. RUNX2 induces *Sp7* expression in preosteoblasts; FGF induces *Runx2* expression and activates RUNX2 in the osteoblast lineage; Wnt induces *Runx2* expression and activates RUNX2, while RUNX2 induces *Tcf7* expression in osteoblast and chondrocyte lineages; RUNX2 induces *Ihh* expression in chondrocytes; and IHH induces *Runx2* expression in osteoprogenitor cells.

into the cartilage [Sun et al., 2001; Zelzer et al., 2001; Himeno et al., 2002; Javed et al., 2005; Conen et al., 2009; Sun et al., 2009]. Osteoblast precursors expressing the reporter gene under the control of *Sp7* promoter, located in the perichondrium prior to vascular invasion of the cartilage, give rise to trabecular osteoblasts, osteocytes, and stromal cells inside the developing bone. Throughout translocation, some precursors were found to intimately associate with invading blood vessels, in pericyte-like fashion [Maes et al., 2010]. Therefore, SP7 may also be involved in the invasion of blood vessels into the developing bone.

FGF SIGNALING AND Runx2

Four fibroblast growth factor receptor (FGFR) genes have been identified in mammals (*FGFR1–4*), each encoding an extracellular domain composed of two or three extracellular immunoglobulin-like (Ig) loops, a transmembrane segment, and an intracellular tyrosine kinase. The affinity and specificity of FGFR1–3 are regulated by tissue-specific alternative splicing, which occurs in the region encoding the carboxyl-terminal half of Ig domain III creating different isoforms, IIIb and IIIc. The receptor isoforms have distinct ligand-binding specificity. For FGFR2, the mesenchymal-based ligands (FGF7 and FGF10) activate only FGFR2b, which is expressed overlying the epithelium, whereas epithelium-based ligands (FGF2, 4, 6, 8, 9) bind to mainly mesenchyme-expressed FGFR2c [Itoh and Ornitz, 2004; Beenken and Mohammadi, 2009; Turner and Grose, 2010]. Signaling from FGFR1, FGFR2, and FGFR3 plays important roles in skeletal development by regulating the proliferation and differentiation of osteoblasts and chondrocytes [Colvin et al., 1996; Deng et al., 1996; Naski et al., 1998; Partanen et al., 1998; Chen et al., 1999; Eswarakumar et al., 2002; Yu et al., 2003; Valverde-Franco et al., 2004; Li et al., 2005; Verheyden et al., 2005; Jacob et al., 2006]. *Fgfr1c*, *Fgfr2b*, *Fgfr2c*, and *Fgfr3c* are expressed in mesenchymal cells in the calvaria [Iseki et al., 1999; Rice et al., 2000; Johnson et al., 2000], and gain of function mutations in *FGFR1*, *FGFR2*, and *FGFR3* genes cause craniosynostosis syndromes, such as Apert, Crouzon, Pfeiffer, and Muenke syndromes and Thanatophoric dysplasia [Cohen, 2004].

Gain-of-function mutations of *Fgfr1* and *Fgfr2* in mice enhanced *Runx2* expression. In mice carrying a P250R mutation in *Fgfr1*, which is orthologous to the Pfeiffer syndrome mutation in humans, premature fusion of calvarial sutures occurred accompanying the marked increase in *Runx2* expression [Zhou et al., 2000]. Mutant mice carrying C342Y replacement in *Fgfr2c*, equivalent to a mutation associated with Crouzon and Pfeiffer syndromes in humans, showed enhanced *Runx2* expression, and *Fgfr2c* loss-of-function mutant mice showed reduced *Runx2* expression [Eswarakumar et al., 2002, 2004]; however, the induction of *Runx2* by *Fgfr2*-activating mutants is controversial in vitro. Overexpression of *Fgfr2c* with or without the S253W mutation equivalent to the Apert mutation enhanced *Runx2* mRNA expression [Tanimoto et al., 2004], whereas overexpression of neither C342Y nor S252W mutant in a murine osteoblastic cell line increased Runx2 mRNA and protein [Mansukhani et al., 2005]. From the findings with Fgfr mutant mice, however, RUNX2 seems to be involved in the pathogenesis of craniosynostosis caused by gain-of-function mutations of FGFRs.

Inactivation of *Fgfr1* in osteo-chondro-progenitor cells resulted in delayed osteoblast differentiation, while inactivation of *Fgfr1* in differentiated osteoblasts accelerated differentiation and increased bone mass [Jacob et al., 2006]. Inactivation of *Runx2* in differentiated osteoblasts also showed similar phenotypes [Maruyama et al., 2007], suggesting that RUNX2 is induced and activated by FGFR1 signaling; however, adult mice with inactivation of *Fgfr1* in osteo-chondro-progenitor cells also showed increased bone mass. Thus, FGFR1 signaling may not be able to induce and activate RUNX2 at the stage of commitment to the osteoblast lineage, because proliferating osteoprogenitor cells express *Fgfr2* but not *Fgfr1*, and *Fgfr1* is upregulated during osteoblast differentiation in calvarial development [Iseki et al., 1999]. Each FGFR seems to have specific and redundant functions at a different stage of osteoblast differentiation through differential expression during osteoblast differentiation.

FGFR signaling also plays an important role in limb development, and gain-of-function mutations of FGFRs cause limb defects [Wilkie et al., 2002]. Apert syndrome, which is a gain-of-function mutation of *FGFR2*, shows limb defects, including broad first digits, complex bony syndactyly, symphalangisms, and radio-humeral or -ulnar synostosis [Wilkie et al., 2002], and osteoblastic cells derived from digital bone show enhanced osteoblast differentiation [Tanimoto et al., 2004]. Pfeiffer syndrome also shows limb defects including broad thumbs, symphalangisms, and radio-humeral or -ulnar synostosis [Wilkie et al., 2002]. *Fgfr2c* mutant mice with the gain-of-function mutation (C342Y) showed multiple joint fusions with excessive bone formation, and *Runx2* expression was upregulated in chondrocytes, the perichondrium, and periosteum

[Eswarakumar et al., 2004]. As the overexpression of *Runx2* in chondrocytes causes multiple joint fusions with excessive bone formation [Ueta et al., 2001], RUNX2 may also be involved in the pathogenesis of limb defects caused by gain-of-function mutations of FGFRs.

FGF2 enhanced *Runx2* mRNA expression through the PKC pathway [Kim et al., 2003]. FGF2 phosphorylated RUNX2 through the MAPK pathway and enhanced transcriptional activity of RUNX2 [Xiao et al., 2002; Ge et al., 2009; Li et al., 2010]; ERK-dependent phosphorylation stabilized RUNX2 protein [Park et al., 2010], and craniosynostosis resulting from *FGFR2* with the S252W substitution was rescued by treatment with U0126, an inhibitor of MEK1/2 [Shukla et al., 2007]. RUNX2 is also activated through the PI3K-Akt pathway [Fujita et al., 2004]. *Fgf2*-deficient mice show decreased bone mass and bone formation, and *Fgf18*-deficient mice show a delay of osteogenic differentiation in the long bones and cranium [Montero et al., 2000; Liu et al., 2002; Ohbayashi et al., 2002]. Although *Runx2* mRNA expression was maintained in *Fgf18*-deficient mice, RUNX2 activity may have been reduced in mice. Thus, FGF2 enhances *Runx2* mRNA expression and activates RUNX2, and FGF18 may have similar activities (Fig. 1).

Wnt SIGNALING AND RUNX2

Canonical Wnt signals are transmitted through stabilizing β-catenin protein by inhibiting GSK3β-mediated β-catenin phosphorylation. Unphosphorylated β-catenin accumulates in the cytoplasm and translocates to the nucleus where it acts as a co-activator with TCF/LEF transcription factors, including TCF7/TCF1, LEF1, TCF7L1/TCF3, and TCF7L2/TCF4 [Sharpe et al., 2001; Hartmann, 2006; Xu and Kimelman, 2007]. *Ctnnb1*/β-*catenin* depletion in osteoblast progenitors using *Prrx1* promoter *Cre* transgenic mice or *Twist2/Dermo1 Cre* knock-in mice and *Ctnnb1* depletion in preosteoblasts using *Sp7* promoter *Cre* transgenic mice result in a complete block of osteoblast differentiation, indicating that *Ctnnb1* is essential for osteoblast differentiation [Day et al., 2005; Hill et al., 2005; Hu et al., 2005; Rodda and McMahon, 2006]. In these conditional knockout mice, *Runx2* is expressed in perichondrial cells surrounding hypertrophic chondrocytes, and these cells differentiate into chondrocytes. *Sp7* is not detected in perichondrial cells whose *Ctnnb1* has been depleted at the osteoblast progenitor stage, whereas *Sp7* is detected in perichondrial cells whose *Ctnnb1* has been depleted at the preosteoblast stage. This result indicates that β-catenin is required for not only *Sp7* expression, but also osteoblast differentiation after the expression of *Sp7*. These findings indicate that RUNX2 is the first transcription factor required for determination of the osteoblast lineage, while SP7 and canonical Wnt signaling further direct the fate of mesenchymal cells to osteoblasts, blocking their differentiation into chondrocytes.

The functions of canonical Wnt signaling in Runx2 expression and activation are still controversial. Wnt signaling enhances *Runx2* expression through the direct binding of TCF7 or LEF1/β-catenin on the *Runx2* promoter and through DNA binding of SMADs and TCF7L2/β-catenin to their cognate sequences as well as protein–protein interactions between them [Gaur et al., 2005; Dong et al., 2006; Rodríguez-Carballo et al., 2010]. Further, FHL2, a member of the LIM-only subclass of the LIM protein superfamily, interacts with β-catenin and potentiates β-catenin nuclear translocation and TCF/LEF transcription, resulting in increased *Runx2* expression [Hamidouche et al., 2008]. In contrast, constitutive active β-catenin and BMP2 synergistically stimulated bone formation without altering RUNX2 protein expression [Mbalaviele et al., 2005]. Wnt activates RUNX2 through activation of the PI3K-Akt pathway, and RUNX2 forms a complex with LEF1 or TCF7l2-TCF7L2 and this complex binds the composite-binding site in the *Fgf18* promoter and activates it [Reinhold and Naski, 2007; Ling et al., 2010], whereas LEF1 interacts with RUNX2 and represses RUNX2-induced activation of the osteocalcin promoter [Kahler and Westendorf, 2003].

The expression patterns of Tcf/Lef family genes in endochondral bones are quite different. *Tcf7* is upregulated in prehypertrophic chondrocytes and is also expressed in perichondrium around hypertrophic chondrocytes and in osteoblasts. *Lef1* is expressed in proliferating chondrocytes, *Tcf7l1* is expressed in the whole cartilage, but *Lef1* and *Tcf7l1* are undetectable in osteoblasts. *Tcf7l2* is expressed in proliferating chondrocytes and is highly expressed in hypertrophic chondrocytes and osteoblasts but not in prehypertrophic chondrocytes [Glass et al., 2005; Hu et al., 2005]. Runx2 is weakly expressed in resting and proliferating chondrocytes and upregulated in prehypertrophic chondrocytes, and is highly expressed in the perichondrium around hypertrophic chondrocytes and in osteoblasts [Inada et al., 1999; Maruyama et al., 2007]. Thus, the expression pattern of *Tcf7* is completely compatible with that in *Runx2*, whereas the expression patterns of *Lef1* and *Tcf7l1* are different from in *Runx2*, and the expression pattern of *Tcf7l2* is partly similar to that of *Runx2*.

Regulation of the expression of Tcf/Lef family genes by RUNX2 was also reported. RUNX2 strongly induced the expression of *Tcf7* and *Lef1* in chondrocyte and osteoblast lineages; the expression of *Tcf7* and *Tcf7l2* was reduced in *Runx2*$^{-/-}$ endochondral skeletons; the expression of *Tcf7*, *Lef1*, and *Tcf7l2* was reduced in *Runx2*$^{-/-}$ calvaria; and RUNX2 enhanced the reporter activity of *Tcf7* promoter [Mikasa et al., 2010]. Combined with the expression patterns of Tcf/Lef family genes and *Runx2*, RUNX2 regulates at least *Tcf7* expression among Tcf/Lef family genes. Further, RUNX2 regulates Lef1ΔN p2 promoter, which is located within the intron between exons 3 and 4 of *Lef1*, and that overexpression of Lef1ΔN in differentiating osteoblasts induces the expression of osteoblast differentiation genes, *osteocalcin* and *Col1a1* [Hoeppner et al., 2009]. Thus, RUNX2 and Wnt signaling are mutually regulated during skeletal development (Fig. 1).

IHH SIGNALING AND RUNX2

In vertebrates, hedgehog signaling is mediated by three GLI transcription factors, GLI1, GLI2, and GLI3, and regulated by several feedback loops. GLI transcription and protein activity are controlled at several different levels by hedgehog signaling. When the levels of the hedgehog ligand are low, GLI2 and GLI3 are processed into truncated N-terminal target repressors. Full-length GLI2 and GLI3 act as target activators, though GLI3 only weakly. High concentra-

tions of the hedgehog ligand downregulate GLI3 at the transcriptional level and also suppress the processing of GLI2 and GLI3 into truncated target repressors. GLI1 is an activator of hedgehog target genes. GLI1 does not undergo proteolytic cleavage and does not function as a repressor [Marigo et al., 1996; Dai et al., 1999; Ruiz and Altaba, 1999; Sasaki et al., 1999; Wang et al., 2000; Ingham and McMahon, 2001]. IHH plays important roles in the regulation of chondrocyte proliferation and differentiation and osteoblast differentiation. *Ihh*-deficient mice show the lack of endochondral bone due to the absence of osteoblasts and *Runx2* expression is absent in the perichondrial cells before vascular invasion [St-Jacques et al., 1999; Hilton et al., 2005]. Further, ectopic expression of *Ihh* in chondrocytes induces *Runx2* expression throughout the perichondrium, although IHH alone is not sufficient to induce bone collar formation throughout it [Long et al., 2004]. Thus, IHH is required for *Runx2* expression in osteoblast precursors during endochondral bone formation but not sufficient for osteoblast differentiation. In $Ihh^{-/-}Gli3^{-/-}$ mice, osteoblast differentiation is partly rescued, because *Runx2* and *Col1a1* are expressed in the perichondrium, although *Sp7* expression and activation of canonical Wnt signaling are still absent in the perichondrium before vascular invasion [Hilton et al., 2005]. The repressor form of GLI3 inhibits DNA binding of RUNX2 [Ohba et al., 2008]. Further, IHH upregulates the expression and function of Runx2 through GLI2 [Shimoyama et al., 2007]. Thus, IHH activates *Runx2* expression by inhibiting the processing of GLI3 to the repressor form and by upregulating the full-length activating form of GLI2. RUNX2 also directly regulates *Ihh* expression in the growth plate [Yoshida et al., 2004]. In the processes of endochondral ossification, RUNX2 plays an important role in chondrocyte maturation, and IHH induces chondrocyte proliferation and inhibits chondrocyte maturation through the induction of parathyroid hormone-related peptide (PTHrP), which inhibits *Runx2* expression through the PKA signaling pathway [Vortkamp et al., 1996; St-Jacques et al., 1999; Enomoto et al., 2000; Takeda et al., 2001; Ueta et al., 2001; Iwamoto et al., 2003; Li et al., 2004]. Thus, in the growth plate, RUNX2 induces chondrocyte maturation and enhances chondrocyte proliferation through the direct induction of *Ihh* expression; IHH induces PTHrP; PTHrP inhibits chondrocyte maturation through the inhibition of *Runx2* expression, forming a negative feedback loop in chondrocyte maturation; and IHH induces *Runx2* expression in the perichondrium and induces osteoblast differentiation (Fig. 1).

CONCLUDING REMARKS

RUNX2 plays essential roles in osteoblast differentiation and chondrocyte maturation, and RUNX2, SP7, and major signaling pathways, including FGFR, Wnt, and IHH, interact in osteoblast differentiation and/or chondrocyte maturation (Fig. 1). However, it remains to be clarified how *Runx2* expression is regulated in the two lineages and how RUNX2 regulates both osteoblast differentiation and chondrocyte maturation at different stages and locations during bone development. Elucidation of the reciprocal regulation of RUNX2, SP7, and the major signaling pathways at an appropriate time and space in bone development would reveal why RUNX2 plays critical roles in both osteoblast differentiation and chondrocyte maturation.

REFERENCES

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

Beenken A, Mohammadi M. 2009. The FGF family: Biology, pathophysiology and therapy. Nat Rev Drug Discov 8:235–253.

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

Chen L, Adar R, Yang X, Monsonego EO, Li C, Hauschka PV, Yayon A, Deng CX. 1999. Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis. J Clin Invest 104:1517–1525.

Cohen MM. 2004. FGFs/FGFRs and associated disorders. In: Epstein CJ, Erickson RP, Wynshaw-Boris A, editors. Inborn errors of development. New York: Oxford University Press. pp 380–400.

Colvin JS, Bohne BA, Harding GW, McEwen DG, Ornitz DM. 1996. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. Nat Genet 12:390–397.

Conen KL, Nishimori S, Provot S, Kronenberg HM. 2009. The transcriptional cofactor Lbh regulates angiogenesis and endochondral bone formation during fetal bone development. Dev Biol 333:348–358.

Dai P, Akimaru H, Tanaka Y, Maekawa T, Nakafuku M, Ishii S. 1999. Sonic hedgehog-induced activation of the Gli1 promoter is mediated by GLI3. J Biol Chem 274:8143–8152.

Day TF, Guo X, Garrett-Beal L, Yang Y. 2005. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8:739–750.

Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. 1996. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell 84:911–921.

Dong YF, Soung do Y, Schwarz EM, O'Keefe RJ, Drissi H. 2006. Wnt induction of chondrocyte hypertrophy through the Runx2 transcription factor. J Cell Physiol 208:77–86.

Enomoto H, Enomoto-Iwamoto M, Iwamoto M, Nomura S, Himeno M, Kitamura Y, Kishimoto T, Komori T. 2000. Cbfa1 is a positive regulatory factor in chondrocyte maturation. J Biol Chem 275:8695–8702.

Eswarakumar VP, Monsonego-Ornan E, Pines M, Antonopoulou I, Morriss-Kay GM, Lonai P. 2002. The IIIc alternative of Fgfr2 is a positive regulator of bone formation. Development 129:3783–3793.

Eswarakumar VP, Horowitz MC, Locklin R, Morriss-Kay GM, Lonai P. 2004. A gain-of-function mutation of Fgfr2c demonstrates the roles of this receptor variant in osteogenesis. Proc Natl Acad Sci USA 101:12555–12560.

Fu H, Doll B, McNelis T, Hollinger JO. 2007. Osteoblast differentiation in vitro and in vivo promoted by Osterix. J Biomed Mater Res A 83:770–778.

Fujita T, Azuma Y, Fukuyama R, Hattori Y, Yoshida CA, Koida M, Ogita K, Komori T. 2004. Runx2 induces osteoblast and chondrocyte differentiation and enhances their migration by coupling with PI3K-Akt signaling. J Cell Biol 166:85–95.

Gaur T, Lengner CJ, Hovhannisyan H, Bhat RA, Bodine PV, Komm BS, Javed A, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2005. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. J Biol Chem 280:33132–33140.

Ge C, Xiao G, Jiang D, Yang Q, Hatch NE, Roca H, Franceschi RT. 2009. Identification and functional characterization of ERK/MAPK phosphorylation sites in the Runx2 transcription factor. J Biol Chem 284:32533–32543.

Glass DA II, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G. 2005. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 8:751–764.

Hamidouche Z, Haÿ E, Vaudin P, Charbord P, Schüle R, Marie PJ, Fromigué O. 2008. FHL2 mediates dexamethasone-induced mesenchymal cell differentiation into osteoblasts by activating Wnt/beta-catenin signaling-dependent Runx2 expression. FASEB J 22:3813–3822.

Hartmann C. 2006. A Wnt canon orchestrating osteoblastogenesis. Trends Cell Biol 16:151–158.

Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C. 2005. Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8:727–738.

Hilton MJ, Tu X, Cook J, Hu H, Long F. 2005. Ihh controls cartilage development by antagonizing Gli3, but requires additional effectors to regulate osteoblast and vascular development. Development 132:4339–4351.

Himeno M, Enomoto H, Liu W, Ishizeki K, Nomura S, Kitamura Y, Komori T. 2002. Impaired vascular invasion of Cbfa1-deficient cartilage engrafted in the spleen. J Bone Miner Res 17:1297–1305.

Hoeppner LH, Secreto F, Jensen ED, Li X, Kahler RA, Westendorf JJ. 2009. Runx2 and bone morphogenic protein 2 regulate the expression of an alternative Lef1 transcript during osteoblast maturation. J Cell Physiol 221:480–489.

Hu H, Hilton MJ, Tu X, Yu K, Ornitz DM, Long F. 2005. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. Development 132:49–60.

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

Ingham PW, McMahon AP. 2001. Hedgehog signaling in animal development: Paradigms and principles. Genes Dev 15:3059–3087.

Iseki S, Wilkie AO, Morriss-Kay GM. 1999. Fgfr1 and Fgfr2 have distinct differentiation-and proliferation-related roles in the developing mouse skull vault. Development 126:5611–5620.

Itoh N, Ornitz DM. 2004. Evolution of the Fgf and Fgfr gene families. Trends Genet 20:563–569.

Iwamoto M, Kitagaki J, Tamamura Y, Gentili C, Koyama E, Enomoto H, Komori T, Pacifici M, Enomoto-Iwamoto M. 2003. Runx2 expression and action in chondrocytes are regulated by retinoid signaling and parathyroid hormone-related peptide (PTHrP). Osteoarthritis Cartilage 11:6–15.

Jacob AL, Smith C, Partanen J, Ornitz DM. 2006. Fibroblast growth factor receptor 1 signaling in the osteo-chondrogenic cell lineage regulates sequential steps of osteoblast maturation. Dev Biol 296:315–328.

Javed A, Barnes GL, Pratap J, Antkowiak T, Gerstenfeld LC, van Wijnen AJ, Stein JL, Lian JB, Stein GS. 2005. Impaired intranuclear trafficking of Runx2 (AML3/CBFA1) transcription factors in breast cancer cells inhibits osteolysis in vivo. Proc Natl Acad Sci USA 102:1454–1459.

Johnson D, Iseki S, Wilkie AO, Morriss-Kay GM. 2000. Expression patterns of Twist and Fgfr1, -2 and -3 in the developing mouse coronal suture suggest a key role for twist in suture initiation and biogenesis. Mech Dev 91:341–345.

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

Kahler RA, Westendorf JJ. 2003. Lymphoid enhancer factor-1 and beta-catenin inhibit Runx2-dependent transcriptional activation of the osteocalcin promoter. J Biol Chem 278:11937–11944.

Kärner E, Unger C, Cerny R, Ahrlund-Richter L, Ganss B, Dilber MS, Wendel M. 2009. Differentiation of human embryonic stem cells into osteogenic or hematopoietic lineages: A dose-dependent effect of osterix over-expression. J Cell Physiol 218:323–333.

Kim HJ, Kim JH, Bae SC, Choi JY, Kim HJ, Ryoo HM. 2003. The protein kinase C pathway plays a central role in the fibroblast growth factor-stimulated expression and transactivation activity of Runx2. J Biol Chem 278:319–326.

Kim YJ, Kim HN, Park EK, Lee BH, Ryoo HM, Kim SY, Kim IS, Stein JL, Lian JB, Stein GS, van Wijnen AJ, Choi JY. 2006. The bone-related Zn finger transcription factor Osterix promotes proliferation of mesenchymal cells. Gene 366:145–151.

Komori T. 2005. Regulation of skeletal development by the Runx family of transcription factors. J Cell Biochem 95:445–453.

Kurata H, Guillot PV, Chan J, Fisk NM. 2007. Osterix induces osteogenic gene expression but not differentiation in primary human fetal mesenchymal stem cells. Tissue Eng 13:1513–1523.

Li TF, Dong Y, Ionescu AM, Rosier RN, Zuscik MJ, Schwarz EM, O'Keefe RJ, Drissi H. 2004. Parathyroid hormone-related peptide (PTHrP) inhibits Runx2 expression through the PKA signaling pathway. Exp Cell Res 299:128–136.

Li C, Xu X, Nelson DK, Williams T, Kuehn MR, Deng CX. 2005. FGFR1 function at the earliest stages of mouse limb development plays an indispensable role in subsequent autopod morphogenesis. Development 132:4755–4764.

Li Y, Ge C, Franceschi RT. 2010. Differentiation-dependent association of phosphorylated extracellular signal-regulated kinase with the chromatin of osteoblast-related genes. J Bone Miner Res 25:154–163.

Ling L, Dombrowski C, Foong KM, Haupt LM, Stein GS, Nurcombe V, van Wijnen AJ, Cool SM. 2010. Synergism between Wnt3a and heparin enhances osteogenesis via a phosphoinositide 3-kinase/Akt/RUNX2 pathway. J Biol Chem 285:26233–26244.

Liu Z, Xu J, Colvin JS, Ornitz DM. 2002. Coordination of chondrogenesis and osteogenesis by fibroblast growth factor 18. Genes Dev 16:859–869.

Long F, Chung UI, Ohba S, McMahon J, Kronenberg HM, McMahon AP. 2004. Ihh signaling is directly required for the osteoblast lineage in the endochondral skeleton. Development 131:1309–1318.

Maehata Y, Takamizawa S, Ozawa S, Kato Y, Sato S, Kubota E, Hata R. 2006. Both direct and collagen-mediated signals are required for active vitamin D3-elicited differentiation of human osteoblastic cells: Roles of osterix, an osteoblast-related transcription factor. Matrix Biol 25:47–58.

Maes C, Goossens S, Bartunkova S, Drogat B, Coenegrachts L, Stockmans I, Moermans K, Nyabi O, Haigh K, Naessens M, Haenebalcke L, Tuckermann JP, Tjwa M, Carmeliet P, Mandic V, David JP, Behrens A, Nagy A, Carmeliet G, Haigh JJ. 2010. Increased skeletal VEGF enhances beta-catenin activity and results in excessively ossified bones. EMBO J 20:424–441.

Mansukhani A, Ambrosetti D, Holmes G, Cornivelli L, Basilico C. 2005. Sox2 induction by FGF and FGFR2 activating mutations inhibit Wnt signaling and osteoblast differentiation. J Cell Biol 168:1065–1076.

Marigo V, Johnson RL, Vortkamp A, Tabin CJ. 1996. Sonic hedgehog differentially regulates expression of GLI and GLI3 during limb development. Dev Biol 180:273–283.

Maruyama Z, Yoshida CA, Furuichi T, Amizuka N, Ito M, Fukuyama R, Miyazaki T, Kitaura H, Nakamura K, Fujita T, Kanatani N, Moriishi T, Yamana K, Liu W, Kawaguchi H, Nakamura K, Komori T. 2007. Runx2 determines bone maturity and turnover rate in postnatal bone development and is involved in bone loss in estrogen deficiency. Dev Dyn 236:1876–1890.

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

Mbalaviele G, Sheikh S, Stains JP, Salazar VS, Cheng SL, Chen D, Civitelli R. 2005. Beta-catenin and BMP-2 synergize to promote osteoblast differentiation and new bone formation. J Cell Biochem 94:403–418.

Mikasa M, Rokutanda S, Komori H, Ito K, Tsang Y, Date Y, Yoshida CA, Komori T. 2010. Regulation of Tcf7 by Runx2 in chondrocyte maturation

and proliferation. J Bone Miner Metab (in press). DOI:10.1007/s00774-010-0222-z

Montero A, Okada Y, Tomita M, Ito M, Tsurukami H, Nakamura T, Doetschman T, Coffin JD, Hurley MM. 2000. Disruption of the fibroblast growth factor-2 gene results in decreased bone mass and bone formation. J Clin Invest 105:1085–1093.

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

Naski MC, Colvin JS, Coffin JD, Ornitz DM. 1998. Repression of hedgehog signaling and BMP4 expression in growth plate cartilage by fibroblast growth factor receptor 3. Development 125:4977–4988.

Ohba S, Kawaguchi H, Kugimiya F, Ogasawara T, Kawamura N, Saito T, Ikeda T, Fujii K, Miyajima T, Kuramochi A, Miyashita T, Oda H, Nakamura K, Takato T, Chung UI. 2008. Patched1 haploinsufficiency increases adult bone mass and modulates Gli3 repressor activity. Dev Cell 14:689–699.

Ohbayashi N, Shibayama M, Kurotaki Y, Imanishi M, Fujimori T, Itoh N, Takada S. 2002. FGF18 is required for normal cell proliferation and differentiation during osteogenesis and chondrogenesis. Genes Dev 16:870–879.

Park OJ, Kim HJ, Woo KM, Baek JH, Ryoo HM. 2010. FGF2-activated ERK mitogen-activated protein kinase enhances Runx2 acetylation and stabilization. J Biol Chem 285:3568–3574.

Partanen J, Schwartz L, Rossant J. 1998. Opposite phenotypes of hypomorphic and Y766 phosphorylation site mutations reveal a function for Fgfr1 in anteroposterior patterning of mouse embryos. Genes Dev 12:2332–2344.

Reinhold MI, Naski MC. 2007. Direct interactions of Runx2 and canonical Wnt signaling induce FGF18. J Biol Chem 282:3653–3663.

Rice DP, Aberg T, Chan Y, Tang Z, Kettunen PJ, Pakarinen L, Maxson RE, Thesleff I. 2000. Integration of FGF and TWIST in calvarial bone and suture development. Development 127:1845–1855.

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

Rodríguez-Carballo E, Ulsamer A, Susperregui AR, Manzanares-Céspedes C, Sánchez-García E, Bartrons R, Rosa JL, Ventura F. 2010. Conserved regulatory motifs in osteogenic gene promoters integrate cooperative effects of canonical Wnt and BMP pathways. J Bone Miner Res 27 [Epub ahead of print]. DOI:10.1002/jbmr.260

Ruiz I, Altaba A. 1999. Gli proteins encode context-dependent positive and negative functions: Implications for development and disease. Development 126:3205–3216.

Sasaki H, Nishizaki Y, Hui C, Nakafuku M, Kondoh H. 1999. Regulation of Gli2 and Gli3 activities by an amino-terminal repression domain: Implication of Gli2 and Gli3 as primary mediators of Shh signaling. Development 126:3915–3924.

Sharpe C, Lawrence N, Martinez Arias A. 2001. Wnt signalling: A theme with nuclear variations. BioEssays 23:311–318.

Shimoyama A, Wada M, Ikeda F, Hata K, Matsubara T, Nifuji A, Noda M, Amano K, Yamaguchi A, Nishimura R, Yoneda T. 2007. Ihh/Gli2 signaling promotes osteoblast differentiation by regulating Runx2 expression and function. Mol Biol Cell 18:2411–2418.

Shukla V, Coumoul X, Wang RH, Kim HS, Deng CX. 2007. RNA interference and inhibition of MEK-ERK signaling prevent abnormal skeletal phenotypes in a mouse model of craniosynostosis. Nat Genet 39:1145–1150.

St-Jacques B, Hammerschmidt M, McMahon AP. 1999. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. Genes Dev 13:2072–2086.

Sun L, Vitolo M, Passaniti A. 2001. Runt-related gene 2 in endothelial cells: Inducible expression and specific regulation of cell migration and invasion. Cancer Res 61:4994–5001.

Sun X, Wei L, Chen Q, Terek RM. 2009. HDAC4 represses vascular endothelial growth factor expression in chondrosarcoma by modulating RUNX2 activity. J Biol Chem 284:21881–21890.

Takeda S, Bonnamy JP, Owen MJ, Ducy P, Karsenty G. 2001. Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice. Genws-Genes Dev 15:467–481.

Tanimoto Y, Yokozeki M, Hiura K, Matsumoto K, Nakanishi H, Matsumoto T, Marie PJ, Moriyama K. 2004. A soluble form of fibroblast growth factor receptor 2 (FGFR2) with S252W mutation acts as an efficient inhibitor for the enhanced osteoblastic differentiation caused by FGFR2 activation in Apert syndrome. J Biol Chem 279:45926–45934.

Tominaga H, Maeda S, Miyoshi H, Miyazono K, Komiya S, Imamura T. 2009. Expression of osterix inhibits bone morphogenetic protein-induced chondrogenic differentiation of mesenchymal progenitor cells. J Bone Miner Metab 27:36–45.

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

Turner N, Grose R. 2010. Fibroblast growth factor signalling: From development to cancer. Nat Rev Cancer 10:116–129.

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

Valverde-Franco G, Liu H, Davidson D, Chai S, Valderrama-Carvajal H, Goltzman D, Ornitz DM, Henderson JE. 2004. Defective bone mineralization and osteopenia in young adult FGFR3$^{-/-}$ mice. Hum Mol Genet 13:271–284.

Verheyden JM, Lewandoski M, Deng C, Harfe BD, Sun X. 2005. Conditional inactivation of Fgfr1 in mouse defines its role in limb bud establishment, outgrowth and digit patterning. Development 132:4235–4245.

Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ. 1996. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science 273:613–622.

Wang B, Fallon JF, Beachy PA. 2000. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. Cell 100:423–434.

Wilkie AO, Patey SJ, Kan SH, van den Ouweland AM, Hamel BC. 2002. FGFs, their receptors, and human limb malformations: Clinical and molecular correlations. Am J Med Genet 112:266–278.

Xiao G, Jiang D, Gopalakrishnan R, Franceschi RT. 2002. Fibroblast growth factor 2 induction of the osteocalcin gene requires MAPK activity and phosphorylation of the osteoblast transcription factor, Cbfa1/Runx2. J Biol Chem 277:36181–36187.

Xu W, Kimelman D. 2007. Mechanistic insights from structural studies of β-catenin and its binding partners. J Cell Sci 120:3337–3344.

Yoshida CA, Yamamoto H, Fujita T, Furuichi T, Ito K, Inoue K, Yamana K, Zanma A, Takada K, Ito Y, Komori T. 2004. Runx2 and Runx3 are essential for chondrocyte maturation and Runx2 regulates limb growth through induction of Indian hedgehog. Genes Dev 18:952–963.

Yu K, Xu J, Liu Z, Sosic D, Shao J, Olson EN, Towler DA, Ornitz DM. 2003. Conditional inactivation of FGF receptor 2 reveals an essential role for FGF signaling in the regulation of osteoblast function and bone growth. Development 130:3063–3074.

Zelzer E, Glotzer DJ, Hartmann C, Thomas D, Fukai N, Soker S, Olsen BR. 2001. Tissue specific regulation of VEGF expression during bone development requires Cbfa1/Runx2. Mech Dev 106:97–106.

Zhou YX, Xu X, Chen L, Li C, Brodie SG, Deng CX. 2000. A Pro250Arg substitution in mouse Fgfr1 causes increased expression of Cbfa1 and premature fusion of calvarial sutures. Hum Mol Genet 9:2001–2008. J Cell Physiol 2009. 221:480–489.