Runx2 and Osteoblasts

Subjects: Biology

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Runx2 is essential for osteoblast differentiation, chondrocyte maturation, and transdifferentiation of terminally differentiated chondrocytes into osteoblasts. During osteoblast differentiation, Runx2 is weakly expressed in uncommitted mesenchymal cells, and its expression is upregulated in preosteoblasts, reaches the maximal level in immature osteoblasts, and is down-regulated in mature osteoblasts. Runx2 enhances the proliferation of osteoblast progenitors by directly regulating Fgfr2 and Fgfr3. Runx2 enhances the proliferation of suture mesenchymal cells and induces their commitment into osteoblast lineage cells through the direct regulation of hedgehog (Ihh, Gli1, and Ptch1), Fgf (Fgfr2 and Fgfr3), Wnt (Tcf7, Wnt10b, and Wnt1), and Pthlh (Pthr1) signaling pathway genes, and Dlx5. Runx2 heterozygous mutation causes open fontanelle and sutures because more than half of the Runx2 gene dosage is required for the induction of these genes in suture mesenchymal cells. Runx2 induces the proliferation of osteoblast progenitors and their differentiation into osteoblasts through reciprocal regulation via major signaling pathways, including Fgf, hedgehog, Wnt, and Pthlh, and transcription factors, including Sp7 and Dlx5. Runx2 also regulates the expression of bone matrix protein genes, including Col1a1, Col1a2, Spp1, and Bglap/Bglap2. Bglap/Bglap2 (osteocalcin) aligns biological apatite parallel to the collagen fibrils, which is important for bone strength, but osteocalcin does not play a role as a hormone in the pancreas, testis, and muscle.

Runx2 hedgehog Wnt Fgfr Pthr1 Sp7 proliferation differentiation cleidocranial dysplasia osteoblast osteocalcin

1. Introduction

Runx2 belongs to the Runx family, which has the DNA-binding domain runt, and consists of Runx1, Runx2, and Runx3 [1]. Runx2 heterodimerizes with Cbfb and acquires enhanced DNA binding ability and protein stability [2][3][4][5][6]. *Runx2*-deficient (*Runx2*$^{-/-}$) mice lack osteoblasts and bone formation, and chondrocyte maturation is markedly inhibited [7][8][9][10]. In osteoblast differentiation, Runx2 is expressed in uncommitted mesenchymal cells, and its expression is upregulated in preosteoblasts, reaches the maximum level in immature osteoblasts, and is down-regulated in mature osteoblasts [11][12]. Runx2 regulates the proliferation of osteoblast progenitors, their commitment to osteoblast lineage cells, and the expression of bone matrix protein genes.

2. Reciprocal Regulation of the Essential Transcription Factors for Osteoblast Differentiation

Hedgehog signaling is essential for osteoblast differentiation in endochondral bone. Hedgehog binding to Ptch relieves the repression of Smo, which ultimately regulates Gli [13]. Hedgehog signaling is required for *Runx2* expression in the perichondrium at the early process of osteoblast differentiation, which is the differentiation of osteoblast progenitors into preosteoblasts [14][15][16][17] (Figure 1). The sources of osteoblasts in primary spongiosa have been demonstrated to be *Sp7*-expressing perichondrial cells and the transdifferentiated osteoblasts from hypertrophic chondrocytes [18][19][20][21]. Runx2 is essential for the differentiation of perichondrial cells into osteoblasts and transdifferentiation of hypertrophic chondrocytes into osteoblasts [8][9][21]. Runx2 directly regulates *Ihh* expression in chondrocytes, osteoblast progenitors, and osteoblasts, as well as *Gli1* and *Ptch1*expression in osteoblast progenitors and osteoblasts [12][22]. Thus, Runx2 and hedgehog signaling regulate each other, and induce osteoblast differentiation (Figure 1).

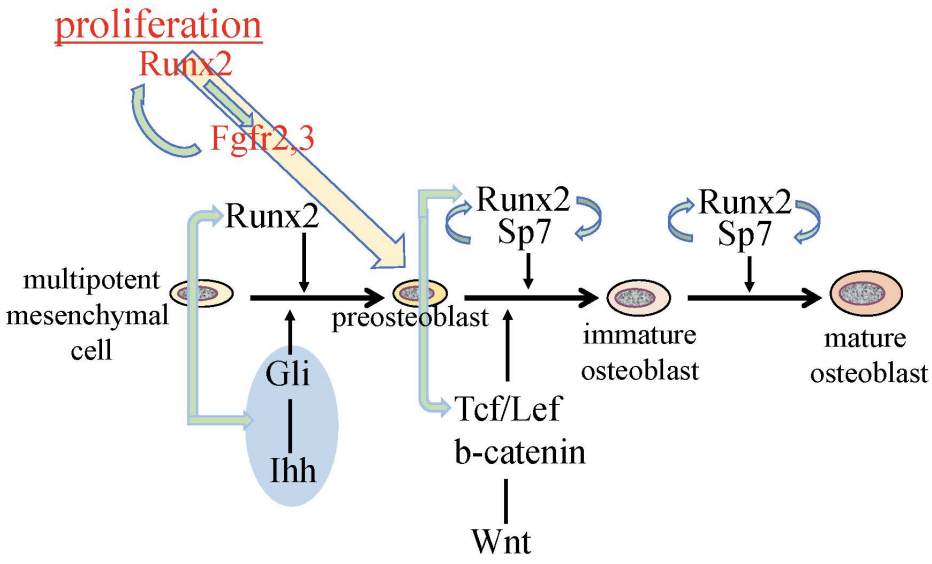

Fig. 1

Figure 1. Regulation of osteoblast proliferation and differentiation by transcription factors. Runx2 induces the differentiation of multipotent mesenchymal cells into preosteoblasts. Ihh is required for the expression of *Runx2* in the perichondrium of endochondral bones. Runx2 induces *Sp7*expression, and Runx2, Sp7, and canonical Wnt signaling induce the differentiation of preosteoblasts into immature osteoblasts. Runx2 and Sp7 are also involved in the maturation of osteoblasts. Runx2 regulates the proliferation of preosteoblasts by inducing *Fgfr2* and *Fgfr3*. *Runx2* expression and that of hedgehog, Fgf, and Wnt signaling pathway genes, and *Sp7* are reciprocally regulated.

Runx2 directly regulates *Sp7* expression, and osteoblasts and bone formation are also absent in *Sp7*$^{-/-}$ mice [23][24]. As Sp7 activates an osteoblast-specific enhancer of *Runx2*, Sp7 is also involved in the regulation of *Runx2* expression [25] (Figure 1). Canonical Wnt signaling is also essential for osteoblast differentiation, because

conditional *Ctnnb1* knockout mice using *Twist2* (*Dermo1*)-Cre, *Col2a1*-Cre, or *Prrx1*-Cre completely lack osteoblasts [26][27][28]. In these mice, *Runx2* is expressed in the perichondrial cells, but *Sp7* expression is weak or absent in perichondrial cells, indicating that osteoblast differentiation is arrested at the stage of preosteoblasts. *Sp7*$^{-/-}$ mice and conditional *Ctnnb1*$^{-/-}$ mice have abundant mesenchymal cells that express Runx2 in the presumptive bone region. As some of the mesenchymal cells in the perichondrium and calvaria differentiate into chondrocytes in *Sp7*$^{-/-}$ mice and conditional *Ctnnb1*$^{-/-}$ mice [26][27][28], Sp7 and canonical Wnt signaling inhibit chondrocyte differentiation and direct Runx2^{+} osteoblast progenitors to become osteoblasts (Figure 1).

3. Regulation of the Proliferation of Osteoblast Progenitors by Runx2

Overexpression of *Runx2* under the control of the *Prrx1* promoter accelerated osteoblast differentiation, inhibited chondrocyte differentiation, and caused limb defects [29]. The limb defects were caused by the ectopic induction of Fgfr1-3 by Runx2 [30]. The expression of Fgfr1-3 was directly regulated by Runx2, and Fgfr2 and Fgfr3 play roles in the proliferation of osteoblast progenitors. The expression of *Fgfr1-3* was markedly reduced in *Runx2*$^{-/-}$ calvaria but not in *Sp7*$^{-/-}$ calvaria. Runx2 increased the proliferation of wild-type osteoblast progenitors and augmented Fgf2-induced proliferation. Thus, Fgf signaling plays a major role in the proliferation of osteoblast progenitors, and Runx2 regulates the proliferation of osteoblast progenitors by inducing *Fgfr2* and *Fgfr3* [30] (Figure 1).

It is difficult to investigate the proliferation of osteoblast lineage cells in vivo because osteoblast lineage cells at different stages of differentiation are mixed and the differentiation stage affects their proliferation. Both *Runx2*$^{-/-}$ mice and *Sp7*$^{-/-}$ mice have cartilaginous skeletons, and lack osteoblasts and bone formation [8][9][24]. *Sp7*$^{-/-}$ mice have abundant mesenchymal cells, which express *Col1a1* weakly and are actively proliferating, in the presumptive bone regions, whereas *Runx2*$^{-/-}$ mice have few mesenchymal cells, which express *Col1a1* at a markedly low level and have low proliferative activity, in the presumptive bone regions [30]. Furthermore, Runx2, Fgfr2, and Fgfr3 are expressed in the mesenchymal cells in *Sp7*$^{-/-}$ mice at levels comparable to those in osteoblasts in wild-type mice. These suggest that mesenchymal cells in *Sp7*$^{-/-}$ mice are preosteoblasts and that *Sp7*$^{-/-}$ mice are an appropriate model for the investigation of preosteoblast proliferation because osteoblast differentiation is blocked at the preosteoblast stage. *Sp7*$^{-/-}$ preosteoblasts proliferated at a similar level as wild-type osteoblast lineage cells in vivo, but they proliferated faster than wild-type osteoblast progenitors in vitro. Fgf2 augmented the proliferation of *Sp7*$^{-/-}$ preosteoblasts, whereas knockdown of *Runx2* inhibited this augmentation and reduced the expression of *Fgfr2* and *Fgfr3*. The amount and proliferation of preosteoblasts in *Sp7*$^{-/-}$ mice was halved in *Sp7*$^{-/-}$*Runx2*$^{+/-}$ mice, indicating that preosteoblast proliferation is dependent on the gene dosage of *Runx2*. Therefore, Runx2 is required for preosteoblast proliferation in vivo, and Runx2 regulates it through the induction of *Fgfr2* and *Fgfr3* [30]. Moreover, Fgf2 enhances the Runx2 capacity for transcriptional activation via the PKC and MAPK pathways [30][31][32][33][34]. Thus, the Fgf signaling pathway and Runx2 positively regulate each other (Figure 1).

4. Molecular Mechanism of the Pathogenesis of Open Fontanelles and Sutures in Cleidocranial Dysplasia (CCD)

Although both intramembranous and endochondral bone development are affected in cleidocranial dysplasia (CCD), the open fontanelles and sutures and hypoplastic clavicles are typical features of CCD [12][35][36]. However, why the development of calvaria and clavicles is the most severely affected in CCD remains unclear.

In *Runx2*$^{+/-}$ mice, the closure of both posterior frontal (PF) and sagittal (SAG) sutures was interrupted. The suture mesenchymal cells expressed Sox9 at a similar level in wild-type and *Runx2*$^{+/-}$ mice. The suture mesenchymal cells also expressed Runx2, but the expression level in *Runx2*$^{+/-}$ mice was half of that in wild-type mice. The cell density and cell proliferation in *Runx2*$^{+/-}$ sutures was less than those in wild-type mice. The expression of hedgehog signaling genes (*Gli1*, *Ptch1*, and *Ihh*), Fgf signaling genes (*Fgfr2 and Fgfr3*), Wnt signaling genes (*Tcf7 and Wnt10b*), *Pth1r, Dlx5, Tnc, and Ncam1* were less in PF and SAG sutures of *Runx2*$^{+/-}$ mice than in those of wild-type mice. Overexpression or knockdown of *Runx2* and ChIP analysis demonstrated that these genes are directly regulated by Runx2 (Figure 2). However, the expression levels of these genes, except Dlx5, were similar in calvarial bone tissues between wild-type and *Runx2*$^{+/-}$ mice. Furthermore, osteoblast marker gene expression was not reduced in the calvarial bone tissues of *Runx2*$^{+/-}$ mice. These findings indicate that more than half of the *Runx2* gene dosage is required for the expression of these Runx2 target genes in suture mesenchymal cells, but half of the *Runx2* gene dosage is sufficient for it in differentiated osteoblasts [12].

In organ culture of *Runx2*$^{+/-}$ calvaria, the ligands or agonists for hedgehog, Fgf, Wnt, and Pthlh signaling pathways enhanced calvarial bone development and suture closure. Furthermore, the antagonists of hedgehog, Fgf, Wnt, and Pthlh signaling pathways inhibited calvarial bone development, suture closure, and proliferation of suture mesenchymal cells in the organ culture of wild-type calvaria. These findings suggested that hedgehog, Fgf, Wnt, and Pthlh signaling pathways are involved in the expansion, condensation, and commitment of suture mesenchymal cells to osteoblast lineage cells, and that Runx2 regulates these processes by inducing their signaling pathway genes [12] (Figure 2).

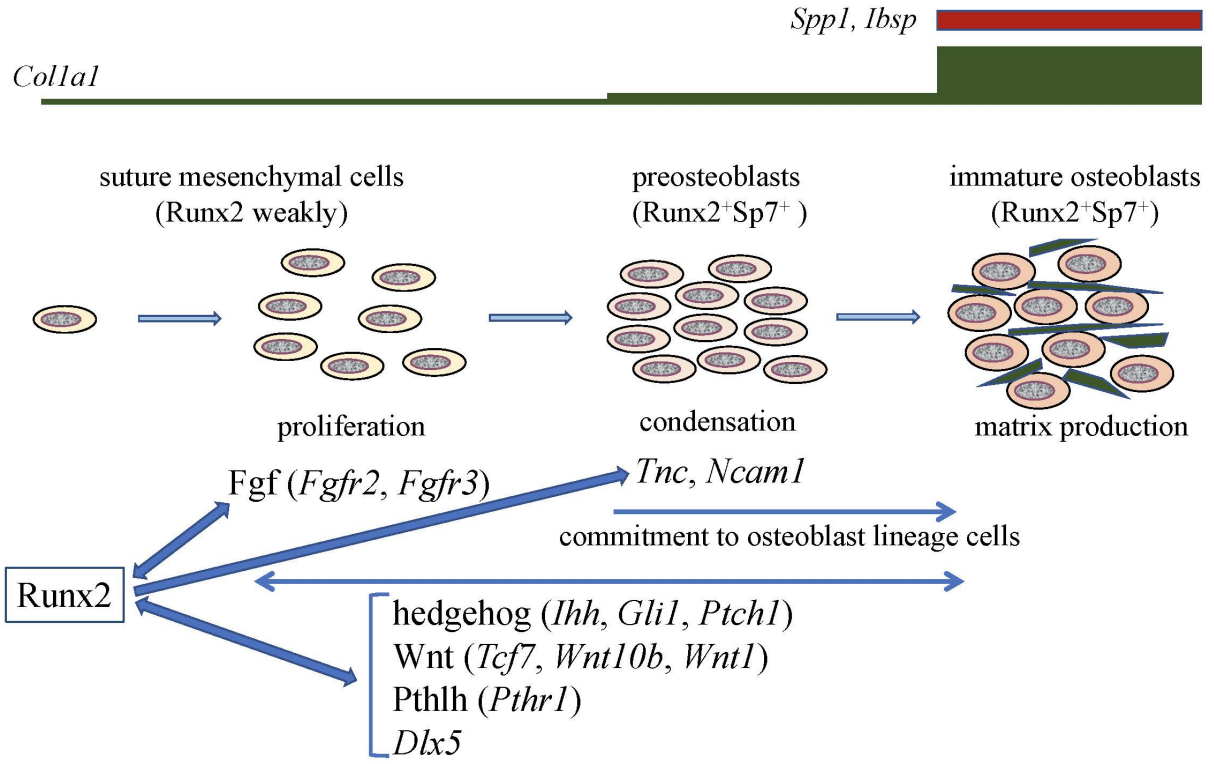

Fig. 2

Figure 2. Calvarial bone development and suture closure. Suture mesenchymal cells weakly express *Runx2*, and its expression is upregulated in preosteoblasts and reaches the maximum level in immature osteoblasts. Runx2 induces *Sp7* expression at the preosteoblast stage. *Col1a1* expression is weak in suture mesenchymal cells, slightly upregulated in preosteoblasts, and markedly upregulated in immature osteoblasts, which also express *Spp1*and *Ibsp*. Runx2 increases the proliferation of suture mesenchymal cells and induces their commitment into osteoblast lineage cells through the induction of Fgf (*Fgfr2* and *Fgfr3*), hedgehog (*Ihh*, *Gli1*, and *Ptch1*), Wnt (*Tcf7*, *Wnt10b*, and *Wnt1*), and Pthlh (*Pthr1*) signaling pathway genes, and *Dlx5*. Fgf signaling plays a role in proliferation, whereas the other genes function in both proliferation and commitment. There is reciprocal regulation between Runx2, and these signaling pathways and Dlx5. In the processes of commitment into osteoblast lineage cells, Runx2 also induces *Tnc* and *Ncam1*, which likely play roles in the condensation of suture mesenchymal cells, and these condensed mesenchymal cells then become preosteoblasts.

Runx2 directly regulates the expression of *Tcf7*, *Wnt10b*, and *Wnt1*, and Tcf7 and Ctnnb1 activate the P1 promoter and osteoblast-specific enhancer of *Runx2* [12][30][37][38]. Thus, Wnt signaling and Runx2 mutually regulate their expression (Figure 1 and 2). As Dlx5 activates the P1 promoter and osteoblast-specific enhancer of *Runx2* [30][39], Dlx5 and Runx2 also mutually regulate their expression (Figure 2). In addition, parathyroid hormone (PTH) increases *Runx2* mRNA, Runx2 protein, and Runx2 activity, PTH induces *Mmp13* promoter activity by activating Runx2 through PKA, and intermittent administration of PTH exerts lower anabolic effects on osteoblast-specific dominant-negative *Runx2* or overexpressing *Runx2* transgenic mice [11][40][41][42]. PTH and Pthlh share a common signaling pathway; therefore, there is also reciprocal regulation between the Pthlh signaling pathway and Runx2 (Figure 2).

5. The Functions of Runx2 in Bone Matrix Protein Gene Expression

After commitment to osteoblastic lineage cells, the osteoblasts express bone matrix protein genes at different levels depending on the maturational stage of the cells. Uncommitted mesenchymal cells weakly express *Col1a1*, its expression is slightly upregulated in preosteoblasts, and is markedly upregulated in immature osteoblasts [12] (Figure 2). Immature osteoblasts express *Spp1* and then *Ibsp*, and mature osteoblasts strongly express *Col1a1* and *Bglap2* [11][43] (Figure 2).

Runx2$^{-/-}$ mice lack osteoblasts, and the expression of bone matrix protein genes, including *Spp1*, *Ibsp*, and *Bglap/Bglap2*, is absent and *Col1a1*expression is very low in the presumptive bone regions [7][8]. Although osteoblasts are observed in type II *Runx2*-specific knockout mice, the expression of *Col1a1*, *Spp1*, and *Bglap/Bglap2* is reduced [44]. In vitro studies also demonstrated that Runx2 is a positive regulator that can upregulate the expression of bone matrix protein genes, including *Col1a1*, *Spp1*, *Ibsp*, *Bglap/Bglap2*, and *Fn1* [45][46][47][48]. Moreover, reporter assays revealed that Runx2 activates the promoters of bone matrix protein genes, including *Col1a1*, *Col1a2*, *Spp1*, and *Bglap/Bglap2* [46][47][49][50].

The functions of osteocalcin (Ocn), which is encoded by *Bglap/Bglap2* that are directly regulated by Runx2, were controversial. Ocn was demonstrated to inhibit bone formation and function as a hormone, which regulates glucose metabolism in the pancreas, testosterone synthesis in the testis, and muscle mass, based on the phenotype of Ocn$^{-/-}$ mice by Karsenty's group [51][52][53][54]. Recently, Ocn$^{-/-}$ mice were newly generated by two groups independently [55][56]. Bone strength is determined by bone quantity and quality. The new Ocn$^{-/-}$ mice revealed that Ocn is not involved in the regulation of bone formation and bone quantity, but that Ocn regulates bone quality by aligning biological apatite (BAp) parallel to the collagen fibrils [56]. Moreover, glucose metabolism, testosterone synthesis and spermatogenesis, and muscle mass were normal in the new Ocn$^{-/-}$ mice [55][56]. Thus, the function of Ocn is the adjustment of growth orientation of BAp parallel to the collagen fibrils, which is important for bone strength to the loading direction of the long bone. However, Ocn does not play a role as a hormone in the pancreas, testis, and muscle (Fig. 3) [57].

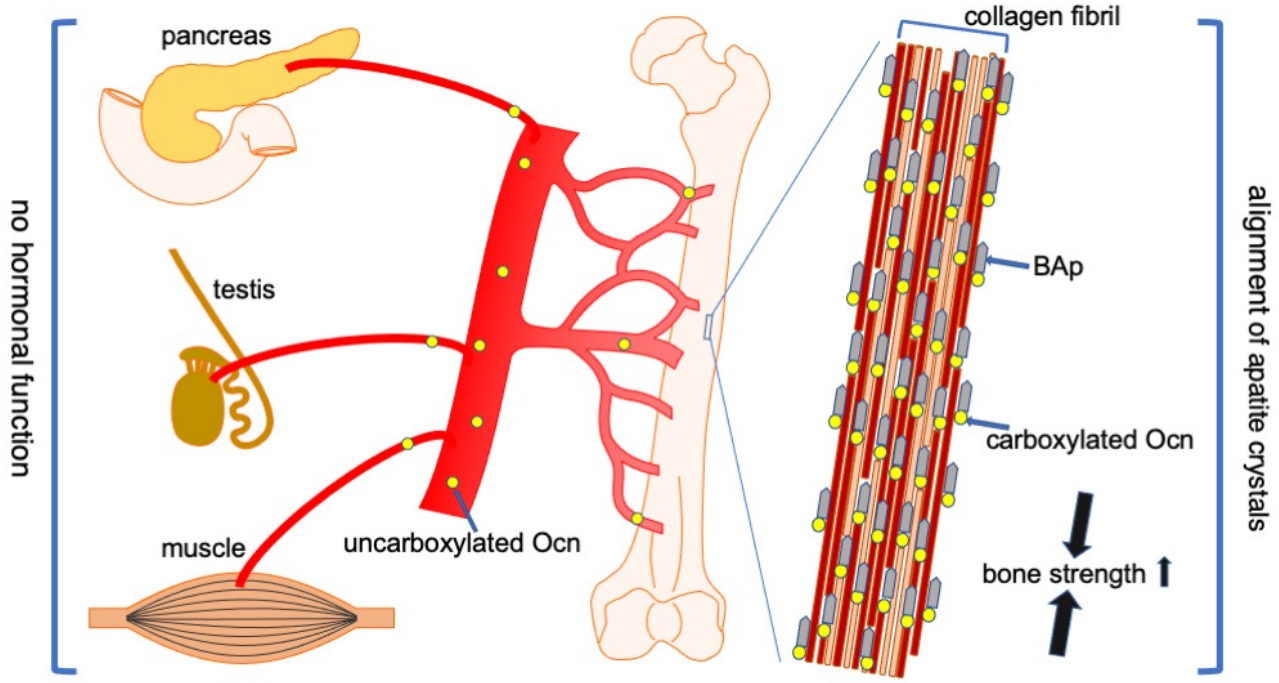

Figure 3. Functions of Ocn in bone, pancreas, testis, and muscle. Carboxylated Ocn is required for the alignment of BAp parallel to the collagen fibers and optimal bone strength. However, two newly generated $Ocn^{-/-}$ mouse lines and $Ocn^{-/-}$ rats [58] did not exhibit the impaired glucose metabolism, reduced testosterone synthesis and spermatogenesis, and reduced muscle mass observed in the $Ocn^{-/-}$ mouse line generated by Karsenty's group. Thus, uncarboxylated Ocn does not physiologically function as a hormone that regulates glucose metabolism in the pancreas, testosterone synthesis in testis, or muscle mass.↑: Bone strength is increased by carboxylated Ocn regulates the alignment of .

6. Conclusions

Runx2 is required for the proliferation of preosteoblasts in whole skeletons and mesenchymal cells in sutures. Indeed, Runx2 is required for the commitment of mesenchymal cells to osteoblast lineage cells. Thus, Runx2 makes a condensed cell layer of uncommitted mesenchymal cells or osteoblast progenitors by increasing their proliferation and facilitates their differentiation into osteoblast lineage cells. Runx2 can exert multiple functions through reciprocal regulation via major signaling pathways, including Fgf, hedgehog, Wnt, and Pthlh, and transcription factors, including Sp7 and Dlx5. Osteoblast proliferation and differentiation are likely regulated by such reciprocal regulation rather than the cascade of transcription factors. Runx2 target protein Ocn regulates the alignment of BAp and is required for bone strength but not for glucose metabolism in the pancreas, testosterone synthesis in testis, or muscle mass.

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