Developmental regulation of the growth plate

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Vertebrates do not look like jellyfish because the bones of their skeletons are levers that allow movement and protect vital organs. Bones come in an enormous variety of shapes and sizes to accomplish these goals, but, with few exceptions, use one process — endochondral bone formation — to generate the skeleton. The past few years have seen an enormous increase in understanding of the signalling pathways and the transcription factors that control endochondral bone development.

Bones are supremely local in their function: they allow adjacent muscles to move them, they move within specialized joint structures, and they protect adjacent organs. But the sizes and shapes of bones need to be carefully coordinated to allow efficient movement of our symmetric bodies. It is not surprising, therefore, that both local paracrine regulators, as well as hormones travelling through the bloodstream, control both bone development and bone remodelling throughout life. The usual suspects — bone morphogenetic proteins (BMPs), Wnts, fibroblast growth factors (FGFs), hedgehog proteins, insulin-like growth factors and retinoids — are essential for normal bone formation. These locally produced factors are joined by systemic factors such as growth hormone, thyroid hormone, oestrogen, androgen, vitamin D and glucocorticoids to control bone growth.

Substantial progress has been made in the past few years in understanding how local signalling molecules, working through key transcription factors, interact and control the growth and differentiation of bones. Here I focus on several intensively studied signalling systems and transcription factors to illustrate the logic of the molecular regulation of bone formation, with an emphasis on how distinct signalling pathways interact to coordinate development.

Endochondral bone formation

Bone formation begins when mesenchymal cells form condensations — clusters of cells that adhere through the expression of adhesion molecules[1] (Fig. 1). In a few areas, most notably the flat bones of the skull, the cells of these condensations differentiate directly into bone-forming osteoblasts. These cells lay down a matrix particularly rich in type I collagen in a process named intramembranous bone formation. But this straightforward process is the exception. In most condensations, the cells become chondrocytes, the primary cell type of cartilage; cells at the border of condensations form a perichondrium. Chondrocytes have a characteristic shape, secrete a matrix rich in type II collagen and the proteoglycan aggrecan, and, more generally, express a characteristic genetic program driven by SOX9 (see below) and other transcription factors.

The cartilage enlarges through chondrocyte proliferation and matrix production (Fig. 1b). Chondrocytes in the centre of the cartilage mould then stop proliferating, enlarge (hypertrophy), and change their genetic program to synthesize type X collagen (Fig. 1c). The hypertrophic chondrocyte, simply through its size, is the principal engine of bone growth[2], and is also a master regulatory cell. Hypertrophic chondrocytes direct the mineralization of their surrounding matrix, attract blood vessels through the production of vascular endothelial growth factor and other factors, and attract chondroclasts (closely related or identical to osteoclasts, which are cells of the macrophage lineage that digest matrix). Hypertrophic chondrocytes direct adjacent perichondrial cells to become osteoblasts; these secrete a characteristic matrix, forming a bone collar (Fig. 1d). Hypertrophic chondrocytes then undergo apoptotic cell death. The cartilage matrix left behind provides a scaffold for osteoblasts that invade the cartilage mould along with blood vessels and lay down a true bone matrix within it (primary spongiosa; Fig. 1e).

While hypertrophic chondrocytes perform these multiple tasks in the centre of the cartilage mould, the mould enlarges further through continued proliferation of chondrocytes (Fig. 1f). As the bone enlarges, haematopoietic stem cells interact with the stroma to establish the main site for haematopoiesis in post-natal life. To varying extents, depending on the identity of the particular bone, a portion of these chondrocytes assume a flattened, discoid shape and form columns like stacks of coins with a clear orientation that directs the lengthening of the bone along one axis more than another. Bone lengthening, particularly rapid during fetal life, is driven primarily by the rate of production of hypertrophic chondrocytes from these proliferating chondrocytes. As bones enlarge further, so-called secondary ossification centres are established when chondrocytes in characteristic, new locations stop proliferating, hypertrophy, and attract vascular invasion along with osteoblasts. In the long bones of the limb, growth chondrocytes continue to proliferate between regions of bone of the primary and secondary ossification centres. This cartilage is then called the growth plate, as it forms a distinct plate of cells between the bone of the secondary ossification centre and the primary spongiosa. At the top of the growth plate, round chondrocytes no longer proliferate rapidly and are called resting or reserve chondrocytes, and probably serve as precursors for the flat proliferating columnar chondrocytes (Fig. 1g). In humans, growth plates disappear at the time of adolescence after a burst of pubertal activity.

Signalling pathways that control bone growth

Ihh/PTHrP signalling

Indian hedgehog (Ihh) is a master regulator of bone development, coordinating chondrocyte proliferation, chondrocyte

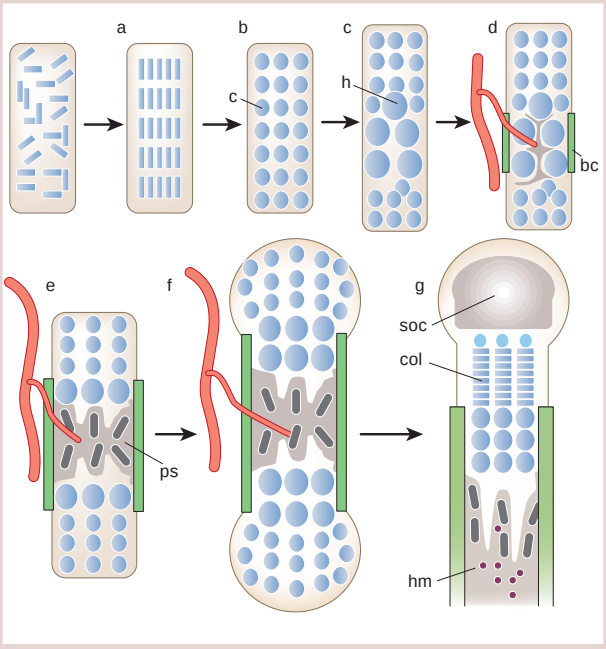

Figure 1 Endochondral bone formation. **a**, Mesenchymal cells condense. **b**, Cells of condensations become chondrocytes (c). **c**, Chondrocytes at the centre of condensation stop proliferating and become hypertrophic (h). **d**, Perichondrial cells adjacent to hypertrophic chondrocytes become osteoblasts, forming bone collar (bc). Hypertrophic chondrocytes direct the formation of mineralized matrix, attract blood vessels, and undergo apoptosis. **e**, Osteoblasts of primary spongiosa accompany vascular invasion, forming the primary spongiosa (ps). **f**, Chondrocytes continue to proliferate, lengthening the bone. Osteoblasts of primary spongiosa are precursors of eventual trabecular bone; osteoblasts of bone collar become cortical bone. **g**, At the end of the bone, the secondary ossification centre (soc) forms through cycles of chondrocyte hypertrophy, vascular invasion and osteoblast activity. The growth plate below the secondary centre of ossification forms orderly columns of proliferating chondrocytes (col). Haematopoietic marrow (hm) expands in marrow space along with stromal cells.

differentiation and osteoblast differentiation. Ihh is a member of the hedgehog family of secreted ligands, and is thus closely related to Sonic hedgehog, one of the main regulators of limb outgrowth (see review in this issue by Mariani and Martin, page 319). During endochondral bone development, Ihh is synthesized by chondrocytes leaving the proliferative pool (prehypertrophic chondrocytes) and by early hypertrophic chondrocytes. Ihh binds to its receptor Patched-1 (Ptc-1); this binding leads to activation of Smoothened (Smo), a membrane protein required for the cellular actions of Ihh. Active Smo then triggers a cascade that leads to gene activation.

Because Ihh action increases expression of Ptc-1, changes in levels of *Ptc-1* messenger RNA offer a convenient assay for evidence of Ihh action at the cellular level. *Ihh*$^{-/-}$ mice have normal bones at the condensation stage, but subsequently develop pronounced abnormalities of bone growth[3]. All cartilage elements are small because of a marked decrease in chondrocyte proliferation. The proliferative effect of Ihh is likely to be a direct action on chondrocytes, because the expression of Ptc-1 and of other general targets of hedgehog signalling is stimulated by Ihh in chondrocytes. Furthermore, cartilage-specific knockout of *Smo* leads to decreased proliferation of chondrocytes, and either chondrocyte-specific transgenic expression of Ihh or of a constitutively active form of Smo increases chondrocyte proliferation[4].

A second abnormality in the bones of *Ihh*$^{-/-}$ mice is an increase in the fraction of chondrocytes that are post-mitotic, hypertrophic chondrocytes. This abnormality results from chondrocytes leaving the pool of proliferating chondrocytes prematurely. They do so because the cartilage in *Ihh*$^{-/-}$ mice fails to synthesize parathyroid hormone-related protein (PTHrP). PTHrP is a protein secreted during fetal life by perichondrial cells at the ends of cartilage moulds and by early proliferative chondrocytes. PTHrP acts on the same G-protein-coupled receptor used by parathyroid hormone, the calcium-regulating hormone. These PTH/PTHrP receptors (PPRs) are expressed at low levels by proliferating chondrocytes and at high levels by prehypertrophic/early hypertrophic chondrocytes.

PTHrP acts primarily to keep proliferating chondrocytes in the proliferative pool. In *PTHrP*$^{-/-}$ or *PPR*$^{-/-}$ mice, chondrocytes become hypertrophic close to the ends of bones[5,6]. Conversely overexpression of PTHrP in chondrocytes[7] or expression of a constitutively active PPR[8] delays the appearance of hypertrophic chondrocytes. As predicted by this model, expression of a constitutively active PPR in chondrocytes of *Ihh*$^{-/-}$ mice reverses the early hypertrophy in these mice (although it has no effect on the defect in chondrocyte proliferation)[9]. The ability of Ihh to stimulate the production of PTHrP and thereby to delay chondrocyte hypertrophy was first shown through viral overexpression of Sonic hedgehog (as an Ihh surrogate) in chick limbs and through addition of Hedgehog protein to mouse limbs *in vitro*[10]. Hedgehog protein blocked hypertrophy of mouse chondrocytes from wild-type limbs but had no effect on chondrocyte differentiation when added to limbs from *PTHrP*$^{-/-}$ or *PPR*$^{-/-}$ mice. These studies demonstrate the obligatory role for PTHrP signalling in mediating the action of Ihh to delay hypertrophy.

The interactions of Ihh and PTHrP led to the hypothesis that these two paracrine factors together control the decision of chondrocytes to leave the proliferative pool through a feedback loop (Fig. 2). PTHrP, secreted from cells near the ends of bone, acts on its receptor on proliferating chondrocytes to keep them proliferating. When chondrocytes are no longer sufficiently stimulated by PTHrP, they stop proliferating and synthesize Ihh. Ihh can then, by mechanisms that are still unknown, stimulate the production of PTHrP at the ends of bone. Experiments using chimaeric mice[11,12] have shown that this feedback loop actually functions in intact bone. Embryonic stem cells missing the gene encoding PPR were inserted into wild-type blastocysts and the phenotypes of the resultant chimaeric bones were analysed. As expected from the phenotype of the *PPR*$^{-/-}$ mice, the *PPR*$^{-/-}$ chondrocytes differentiate into hypertrophic chondrocytes close to the ends of the chimaeric bones. The Ihh formed by these abnormal chondrocytes is, therefore, synthesized much closer to the ends of the bone than normal. This leads to an upregulation of PTHrP expression and a consequent lengthening of the columns of normal proliferative chondrocytes. If the embryonic stem cells are missing both the *PPR* and the *Ihh* genes, then premature hypertrophy of the mutant chondrocytes still occurs, but the *PTHrP* mRNA levels are not changed and the chondrocyte columns formed by the wild-type cells are of normal length.

These studies demonstrate the physiological importance of the interactions of PTHrP and Ihh to determine the lengths of proliferative columns in individual bones. These interactions probably also serve to assure optimum coordination between differentiation patterns of individual cartilage columns within a growing bone. In the absence of PTHrP, the border between proliferating and hypertrophic cells is ragged, with some mixing of proliferating and hypertrophic cells. In the wild-type bone, the sharpness of the transition between proliferation and hypertrophy is probably increased by local feedback between Ihh and PTHrP production.

A third striking abnormality of *Ihh*$^{-/-}$ mice is the absence of osteoblasts in either the primary spongiosa or the bone collar of bones formed by endochondral development[3]. Normally, the first perichondrial cells to convert to osteoblasts are adjacent to the Ihh-producing prehypertrophic/hypertrophic cells of the growth cartilage. Because these cells normally express Ptc-1 in response to Ihh, the effect of Ihh to convert perichondrial cells to osteoblasts is probably a direct one. This hypothesis is further supported by the results of the chimaeric experiments in which *PPR*$^{-/-}$ chondrocytes

mix with wild-type chondrocytes[11,12]. Ectopic osteoblast formation occurs adjacent to the ectopically positioned hypertrophic chondrocytes. No such ectopic bone forms adjacent to the ectopically positioned *PPR*$^{-/-}$;*Ihh*$^{-/-}$ hypertrophic chondrocytes in parallel experiments. Thus, Ihh controls the differentiation of osteoblasts and designates the location at which this differentiation occurs. Although Ihh is absolutely required for osteoblast formation in endochondral bone formation, Ihh is not required for formation of osteoblasts in the bones of the skull that form without a cartilage mould, although growth of intramembranous bone of the skull is apparently slowed in *Ihh*$^{-/-}$ mice[3].

Thus, Ihh is a master regulator of both chondrocyte and osteoblast differentiation during endochondral bone formation. Ihh stimulates chondrocyte proliferation directly and, through stimulation of PTHrP synthesis, determines the distance from the end of the bone at which chondrocytes stop proliferating and undergo hypertrophic differentiation. This is also the site at which Ihh stimulates formation of the bone collar.

Fibroblast growth factor signalling

Recent genetic studies have shown that FGF signalling crucially regulates chondrocyte proliferation and differentiation. Many of the 22 distinct FGF genes and four FGF receptor genes are expressed at every stage of endochondral bone formation[13]. The probable redundancy of ligand utilization combined with the multiple early effects of FGF receptors in development of bone and other tissues have made genetic analysis of the roles of FGF signalling during bone development a particular challenge. In the earliest stages of endochondral bone development, FGF receptor-2 (FGFR2) is expressed in condensing mesenchyme. Multiple FGFs are expressed in condensations and surrounding mesenchyme, although their roles are not established. One possible role of FGF signalling at this early stage is suggested by the finding that FGFs can stimulate SOX9 expression (see below) in a cultured mesenchymal cell line[14].

As chondrocytes form, proliferating chondrocytes express FGFR3 and prehypertrophic/hypertrophic chondrocytes express FGFR1. Perichondrial cells express FGFR2 (Fig. 3). Each of these receptors has distinct and important roles in bone development; FGFR3 is best understood. Knockout of *Fgfr3* in mice leads to an increased rate of proliferation of chondrocytes and an expansion of the length of chondrocyte columns[15,16]. Activating point mutations in *Fgfr3* that are found in human chondrodystrophies decrease the rate of proliferation of chondrocytes and lead to shortened, disorganized columns in transgenic mice[17].

Thus, FGF signalling through FGFR3 inhibits proliferation; this inhibition is at least partly through activation of the Janus kinase–signal transducer and activator of transcription-1 (JAK–STAT1) pathway[18]. The ligands responsible for this signalling are not fully defined, although the recent observation that knockout of *Fgf18* leads to an increase in chondrocyte proliferation that closely resembles the effect of *Fgfr3* knockout argues strongly that FGF18 is one such relevant ligand[19,20]. The phenotype of the *Fgf18*-knockout mouse is more severe than that of the *Fgfr3* knockout, in that ossification is delayed in *Fgf18*$^{-/-}$ mice. This delay may reflect lack of activation of Fgfr1 in hypertrophic chondrocytes and perhaps of Fgfr2 and -1 in perichondrium (Fig. 3). Studies of bone explants *in vitro* demonstrate that FGF signalling, independent of effects on Ihh and PTHrP, accelerates terminal differentiation of hypertrophic chondrocytes[21].

From the previous discussion of the Ihh/PTHrP signalling system, one might not expect that simply increasing or decreasing the rate of proliferation of chondrocytes through changes in FGF signalling would also change the lengths of columns of proliferating chondrocytes. No matter what the rate, one might expect that Ihh would change the expression of PTHrP until columns reached their characteristic length. However, knockout of the *Fgfr3* gene increases Ihh expression and activation of FGFR3 decreases Ihh expression[13,17]. Studies *in vitro*, in which levels of Ihh and PTHrP can be regulated independently, support the idea that part of the effects of FGF signalling is mediated by suppression of Ihh expression[21]. Thus, FGF signalling shortens proliferative columns both by decreasing chondrocyte proliferation directly and by suppressing Ihh expression.

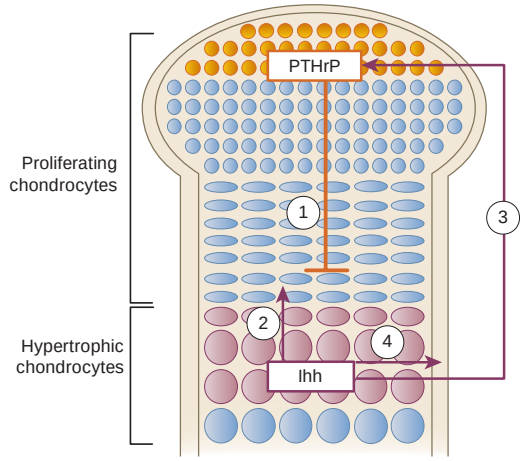

Figure 2 Indian hedgehog (Ihh)/parathyroid hormone-related protein (PTHrP) negative-feedback loop. PTHrP is secreted from perichondrial cells and chondrocytes at the ends of long bones (1). PTHrP acts on receptors on proliferating chondrocytes to keep the chondrocytes proliferating and, thereby, to delay the production of Ihh. When the source of PTHrP production is sufficiently distant, then Ihh is produced. The Ihh acts on its receptor on chondrocytes to increase the rate of proliferation (2) and, through a poorly understood mechanism, stimulates the production of PTHrP at the ends of bones (3). Ihh also acts on perichondrial cells to convert these cells into osteoblasts of the bone collar (4).

Bone morphogenetic protein signalling

BMPs, also called growth and differentiation factors (GDFs), have multiple roles during bone formation. As with the FGF system, the large number of ligands and receptors, as well as the vital roles of many components of this network during early development, has precluded a straightforward use of knockout mice to characterize the roles of BMPs in bone development. Nevertheless, it is clear that BMPs/GDFs have essential roles at every stage of endochondral bone development.

BMPs and GDFs are members of the TGF-β family of paracrine factors that activate heterodimeric receptors with serine/threonine kinase activity[22]. They were discovered because of their remarkable ability to induce endochondral bone formation when injected subcutaneously in mice. The type 1 receptor BMPR1B is expressed in cartilage condensations and the type 1 receptor BMPR1A is expressed broadly in embryonic mesenchyme. An important role for BMP signalling in formation of mesenchymal condensations is supported by the suppression of formation of condensations when the BMP antagonist, Noggin, is expressed in early chick limbs[23] and by the enlarged cartilage primordial in *Noggin*$^{-/-}$ mice[24]. Several condensations are either abnormal or absent in short ear mice, which harbour various inactivating mutations of the *BMP5* gene[25]. Mice missing the gene encoding GDF5 or BMPR1B have abnormalities in digit formation that involve failure of extension of condensations that lead to digit formation[26–28]. Understanding of the full role of BMPs and their receptors during condensation formation, however, will require further studies that generate mice with multiple deficiencies of BMPs, their receptors, and the plethora of BMP signalling modulators specifically in early condensations.

BMPs have multiple important roles during later stages of cartilage development. BMP2, -3, -4, -5 and -7 are expressed in

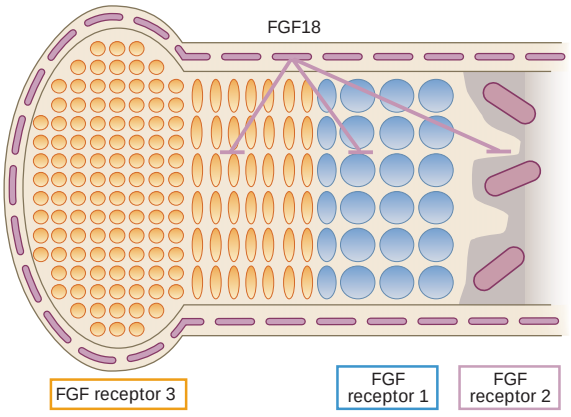

Figure 3 Fibroblast growth factor (FGF) signalling in the growth plate. FGF receptor 3 (FGFR3; yellow) is expressed in proliferating chondrocytes, FGFR1 (light blue) is expressed in prehypertrophic and hypertrophic chondrocytes and perichondrium (not shown), and FGFR2 (purple) is expressed in the perichondrium, periosteum and the primary spongiosa. FGF18 is expressed in the perichondrium and the phenotype of its knockout suggests that it acts on FGFR3 to decrease chondrocyte proliferation and, perhaps, on FGFR1 to delay terminal differentiation of hypertrophic chondrocytes and on FGFR1 and -2 to delay osteoblast development. FGF7, -8 and -17 (not shown) are also expressed in the perichondrium, although knockout of these genes either has no phenotype or leads to early fetal demise, so their function in the growth plate is unknown.

perichondrium, BMP2 and -6 are expressed in hypertrophic chondrocytes, and BMP7 is expressed in proliferating chondrocytes. GDF5, -6 and -7 are expressed at sites of subsequent joint formation. Addition of BMPs to bone explants increases proliferation of chondrocytes and Noggin blocks chondrocyte proliferation[21,29]. Furthermore, addition of BMP2 delays terminal differentiation of hypertrophic chondrocytes, as indicated by expression of osteopontin, and Noggin hastens terminal hypertrophic differentiation[21,29]. These actions of BMPs, established using various overexpression systems, need further confirmation with experiments that knock out selective genes.

BMP signalling increases the expression of Ihh by prehypertrophic chondrocytes[21,29], and so can increase both the proliferation of chondrocytes and the length of proliferating columns of chondrocytes. In both of these actions BMPs oppose the effects of FGF signalling to decrease chondrocyte proliferation and to decrease Ihh expression. Because BMPs and FGFs also have opposite effects on terminal hypertrophic chondrocyte differentiation, these pathways can be considered as antagonizing each other at several levels[21] (Fig. 4). The molecular mechanisms of BMP/FGF antagonism are unknown, although it does not involve simple suppression of ligand expression, as FGF2 increases BMP7 expression and BMP7 increases FGF18 expression.

Transcription factors central to growth plate function

SOX9

SOX9 is essential for converting cells of condensations into chondrocytes and acts further at every stage of chondrocyte differentiation. SOX9 is closely related to the Y-chromosome-encoded testis-determining factor SRY (the abbreviation 'SOX' refers to the DNA-binding SRY box found in SOX-family members) and other DNA-binding proteins in the HMG (high mobility group) family. SOX9 is expressed in cells of mesenchymal condensations and in proliferating chondrocytes, but not in hypertrophic chondrocytes. In cultured cells, SOX9 stimulates transcription of a number of cartilage matrix genes, including *Col2a1*, *Col11a2* and *aggrecan*. In transgenic mice, ectopic expression of *Sox9* leads to expression of *Col2a1* in brain.

The essential role for SOX9 in chondrocyte formation was demonstrated by analysis of chimaeric mice containing descendants of $Sox9^{-/-}$ embryonic stem cells in wild-type hosts[30]. Mesenchymal cells of the $Sox9^{-/-}$ lineage could not participate in condensations or subsequent formation of chondrocytes. When a Cre-*loxP* strategy (a powerful technology that allows target genes to be spliced out of specific cells and tissues) was used to knock *Sox9* out selectively from early limb mesenchyme, no cartilage condensations formed and increased apoptosis in the mesenchyme was noted[31]. Thus, Sox9 may direct differentiation and thereby allow survival of cells in condensations.

When Sox9 was deleted from chondrocytes at later stages of development, through the use of Cre driven by a *Col2a1* promoter, the resultant chondrocytes displayed decreased proliferation, decreased expression of matrix genes, and decreased expression of elements of the Ihh–PTHrP signalling pathways. These phenotypes closely resemble those of mice missing both Sox5 and Sox6, other Sox-family members that cooperate with Sox9 in regulating many chondrocyte genes[32] and which are not expressed in the absence of Sox9[31]. Even heterozygous deletion of *Sox9* in mice leads to cartilage hypoplasia and a perinatal lethal osteochondrodysplasia that resembles campomelic dysplasia, a human syndrome caused by haploinsufficiency of *SOX9*[31]. These mice have fewer chondrocytes than normal in all bone anlage and malformation of subsequent bones. Furthermore, proliferative chondrocytes seem to convert prematurely to hypertrophic chondrocytes, which then mineralize their matrix prematurely. Thus, SOX9 is critical for all phases of the chondrocyte lineage from early condensations to the conversion of proliferating chondrocytes to hypertrophic chondrocytes.

Runx2

Runx2 (previously called Cbfa1) came to prominent attention because mice missing Runx2 have no osteoblasts[33,34]. It is also important in the growth plate, as it drives proliferative chondrocytes to differentiate further into hypertrophic chondrocytes. Runx2 is a member of a family of transcription factors that share the DNA-binding domain of the *Drosophila* pair rule gene *runt*. Runx2 is expressed in the late condensation stage of chondrogenesis and then has substantially decreased expression in proliferating chondrocytes, with increased expression again in prehypertrophic and hypertrophic chondrocytes. It is highly expressed in perichondrial cells and in osteoblasts.

Mice missing Runx2 have no osteoblasts and also exhibit abnormalities of chondrocyte maturation[33–35]. Most bones either lack or have decreased numbers of hypertrophic chondrocytes, and the hypertrophic chondrocytes that are present fail to mineralize their matrix and have decreased or absent expression of genes such as *osteopontin* and *matrix metalloproteinase 13*, which are normally expressed by late hypertrophic chondrocytes. These abnormalities can be reversed by transgene-driven expression of Runx2 in chondrocytes[36,37]. Transgenic expression of Runx2 in wild-type mice accelerates hypertrophy of normal chondrocytes and even induces hypertrophy and subsequent bone formation in cartilage that normally never undergoes hypertrophy, such as the cartilage of tracheal rings. Transgenic expression of a dominant-negative from of Runx2 blocks hypertrophy of all chondrocytes[37]. The hypertrophy that occurs in the absence of Runx2 may result from the action of other Runx-family members, such as Runx3.

Prospects

In this brief overview, the focus on a small number of interacting signalling systems and transcription factors only begins to analyse the number of important signals that determine endochondral bone formation. As noted earlier, growth plate development is affected by growth hormone and insulin-like growth factors, Wnts, growth

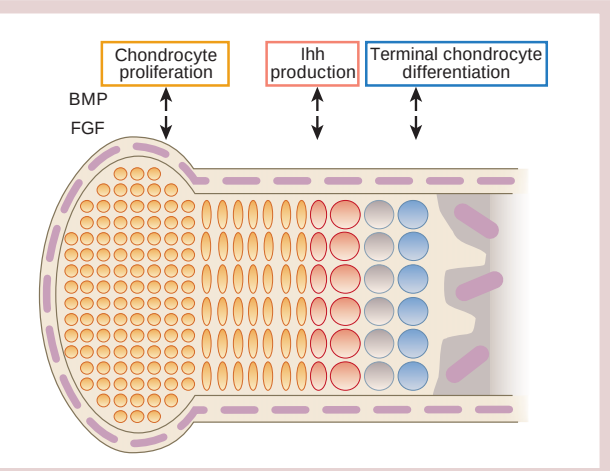

Figure 4 Opposing actions of bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) on the growth plate. FGFs act to decrease chondrocyte proliferation, to increase the production of Ihh (and, thus, the production of Ptc-1 and PTHrP), and to accelerate the differentiation of hypertrophic chondrocytes into terminally differentiated chondrocytes that express osteopontin and other characteristic markers. BMPs act at each of these steps in a manner opposite to that of FGFs.

factors, cytokines, and nuclear receptors responsive to retinoids, thyroid hormone, oestrogen, androgen, vitamin D and glucocorticoids, among others. Furthermore, even with the best-characterized signalling systems, the crucial target transcription factors that mediate the actions of these signals are poorly understood.

More generally, many important biological phenomena described here have no clear molecular explanation. The determinants of the polarity of proliferative columns — both the orientation of the columns within the bone and the asymmetry associated with hypertrophic differentiation occurring at one characteristic end of columns — are not known. The control of the profound and progressive slowing of bone growth in late fetal and then early post-natal life is not understood. The pathways used to determine the enormous variety of specific bone shapes and sizes that all arise using the common endochondral development process are unknown. The determinants of chondrocyte shape — small and round becoming flat and then hypertrophic — are unknown, but crucial for an understanding of bone lengthening. The coordination of growth plate function with the development of joints, tendons and ligaments is under study, but our understanding remains preliminary. The signals that bring haematopoietic stem cells to bone marrow have not yet been clarified. Fortunately, the increasingly powerful tools now available make all of these questions accessible to investigation. □

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