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Multiple Functions of BMPs in Chondrogenesis

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Abstract The ability of bone morphogenetic proteins (BMPs) to promote chondrogenesis has been investigated extensively over the past two decades. Although BMPs promote almost every aspect of chondrogenesis, from commitment to terminal differentiation is well known, the mechanisms of BMP action in discrete aspects of endochondral bone formation have only recently begun to be investigated. In this review, we focus on in vivo studies that have identified interactions between BMP signaling pathways and key downstream targets of BMP action in chondrogenesis. We also discuss evidence regarding the potential roles of BMP receptors in mediating distinct aspects of chondrogenesis, and studies investigating the intersection of BMP pathways with other pathways known to coordinate the progression of chondrocytes through the growth plate. These studies indicate that both Smad-dependent and -independent BMP pathways are required for chondrogenesis, and that BMPs exert essential roles via regulation of the Indian hedgehog (IHH)/parathyroid hormone-related protein (PTHrP) and fibroblast growth factor (FGF) pathways in the growth plate. J. Cell. Biochem. 93: 93–103, 2004.

Key words: BMP; chondrocyte; chrogenesis; cartilage

The bone morphogenetic protein (BMP) family of secreted growth factors forms a subgroup of molecules within the transforming growth factor β (TGFβ) superfamily. BMPs were identified by virtue of their ability to promote ectopic cartilage and bone formation [Wozney, 1989], and mechanisms underlying this unique capability have been the subject of intense investigation for the last two decades. A molecular understanding of BMP action in chondrogenesis could not be achieved until the framework of the BMP signal transduction pathway had been elucidated [reviewed in Derynck and Zhang, 2003]. Other key insights came from demonstrations that BMPs directly regulate the expression of several chondrocyte-specific genes, and that certain members of the Sox transcription factor family are essential regulators of chondrocyte commitment and differentiation, allowing mechanistic investigations of the connection between BMP signaling, and chondrogenesis. Finally, the use of genetically modified mice has led to novel insights into how signaling pathways activated by BMPs intersect with other pathways to control multiple aspects chondrogenesis. The stratified architecture of the developing growth plate makes it an ideal system for understanding how multiple BMP signaling pathways intersect with other pathways to control specific aspects of cell proliferation, differentiation, survival, and gene expression.

At least two distinct pathways mediate BMP signaling: the canonical Smad pathway and a mitogen-activated protein kinase (MAPK) pathway [reviewed in Derynck and Zhang, 2003]. BMPs transduce signals through the formation of heteromeric complexes of types I and II serine/threonine kinase receptors (Fig. 1). Upon BMP binding, type II receptors phosphorylate

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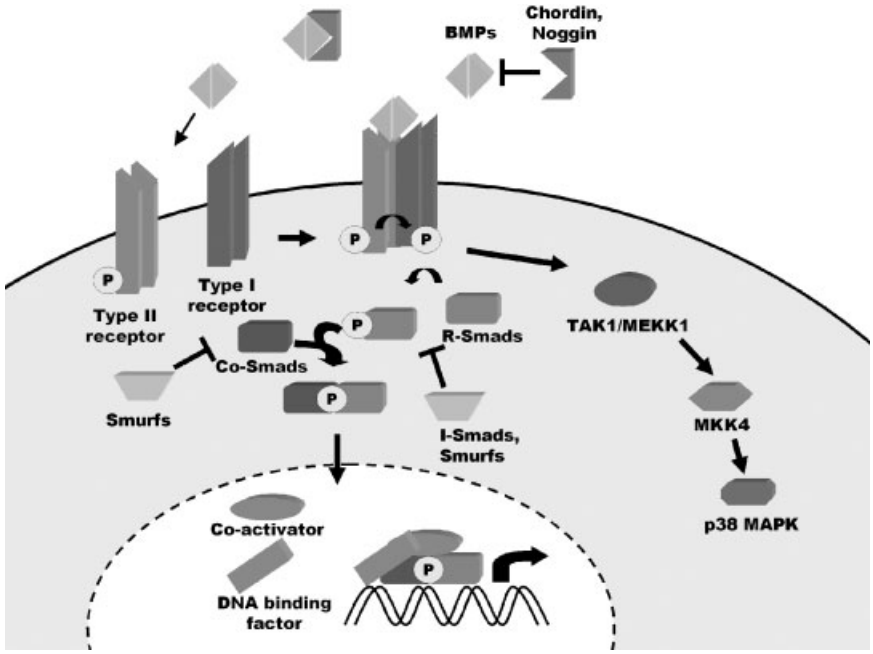

Fig. 1. Mechanism of BMP signaling. Two downstream mediators of BMP signaling have been identified in chondrocytes: The canonical Smad pathway and the p38 MAPK pathway. BMPs signal by binding to a heteromeric receptor complex, leading to the phosphorylation and activation of R-Smads (Smad1, -5, and -8). Phosphorylated R-Smads form a complex with Co-Smads, and this Smad complex translocates into the nucleus to regulate target genes. The binding of BMPs to their receptor complex also leads to the activation of p38 MAPK through TAK1. BMP signaling is regulated by extracellular antagonists (Chd and Nog), and intracellular inhibitors (I-Smads and Smurfs) that target Smads for ubiquitination.

serine/threonine residues in type I receptors. Three type I receptors transduce BMP signals: BMPRIA, BMPRIB, and ALK-2. Activated type I receptors phosphorylate and thereby activate receptor-regulated Smads (R-Smads). Subsequently, R-Smads recruit and bind common-partner Smads (Co-Smads) to form heteromeric complexes. These Smad complexes enter the nucleus and bind DNA directly, or interact with DNA binding proteins to regulate the transcription of target genes. BMPs can also signal by activating TGFβ activated kinase 1 (TAK1). TAK1 leads to the activation of p38 MAPKs. The aspects of BMP signaling in chondrocytes mediated by p38 MAPKs are unclear.

A considerable degree of regulation of BMP signaling occurs at the level of ligand availability. Noggin and Chordin complex with BMPs and prevent them from binding their receptors; in turn, the activities of many BMP antagonists are regulated by post-translational mechanisms [reviewed in Derynck and Zhang, 2003]. Additional levels of control include the modulation of BMP receptor and R-Smad stability via ubiquitin-mediated proteolysis involving the actions of inhibitor Smads (I-Smads) and Smurfs. Finally, there is a considerable degree of crosstalk between BMP and other signaling pathways [von Bubnoff and Cho, 2001; Lyons and Delot, 2003]. For the most part the physiological relevance of these modulatory mechanisms is not well understood in chondrogenesis and will not be discussed here. We will discuss evidence implicating BMP crosstalk with other signaling pathways in the regulation of the growth plate.

The majority of the skeletal system forms through endochondral ossification, in which mesenchymal cells condense and differentiate into chondrocytes, which subsequently form the growth plate, a cartilaginous template for bone formation [reviewed in Kronenberg, 2003]. Growth plate chondrocytes undergo a highly organized differentiation program. Cells at the ends of skeletal elements form the resting or reserve zone, and may provide a source of stem cells. Cells exiting the resting zone form the proliferative compartment. These cells produce cartilage extracellular matrix (ECM) components. Cell–ECM interactions lead to cell shape changes that orient planes of cell division, generating flattened cells that align into columns as they proliferate. These cells eventually exit the cell cycle and differentiate into prehyper-

trophic chondrocytes, which terminally differentiate to form the hypertrophic zone. Cells in the hypertrophic zone become enlarged and undergo apoptosis. The hypertrophic cartilage ECM and associated growth factors promote the invasion of blood vessels and osteoblasts, which then form the bone matrix [Olsen et al., 2000; Kronenberg, 2003].

BMPs in Commitment to the Chondrogenic Lineage

In vitro systems have been particularly informative for investigating the role of BMP pathways in the earliest stages of chondrogenesis: commitment and condensation. BMPs induce differentiation of pluripotent mesenchymal cell lines, such as C3H10T1/2, into chondrocytes when grown at high density [Denker et al., 1999; Haas and Tuan, 1999; Ju et al., 2000; Kramer et al., 2000; Majumdar et al., 2001]. High-density culture mimics the condensation event that precedes chondrogenesis in vivo. One mechanism by which BMPs induce chondrogenesis in this system is through upregulation of N-cadherin (N-cad) function and expression, indicating that one of the earliest roles of BMPs is to promote cell–cell interactions. As expected from the importance of cell–cell interactions in chondrogenesis, N-cad inhibitors neutralize the effects of BMPs [Haas and Tuan, 1999] (Fig. 2).

The requirement for BMP pathways in the formation of precartilaginous condensations is demonstrated in vivo by studies in chick limbs. The use of the secreted BMP inhibitor noggin has been a particularly informative approach; application of Noggin permits antagonism of endogenously produced BMPs while avoiding potential artifacts arising from overexpression of dominant-negative (DN) receptors. Overexpression of noggin blocks condensation, leading to a total absence of cartilage. Overexpression of constitutively active (CA) BMP receptors results in expansion of cartilage at the expense of muscle and soft tissues [Capdevila and Johnson, 1998; Pizette and Niswander, 2000]. These and related studies establish that BMP signaling is required for, and acts as part of, an instructive signal to promote commitment to the chondrogenic lineage.

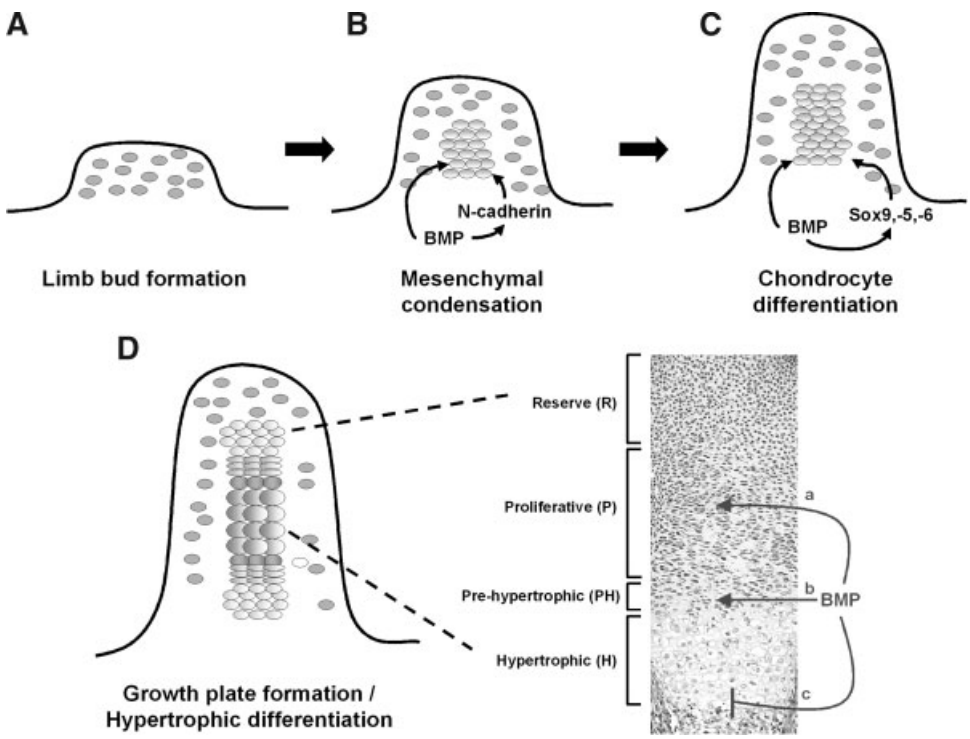

Fig. 2. BMP actions during chondrogenesis. Multiple effects of BMP signaling on chondrogenesis have been demonstrated in vitro and in vivo. **A**: Development of the limb bud. **B**: BMP signaling promotes mesenchymal condensation, in part by promoting N-cadherin production. **C**: BMPs promote chondrocyte differentiation by maintaining expression of Sox transcription factors. **D**: BMPs also have multiple roles during chondrogenesis in the growth plate. They promote proliferation (**a**) and hypertrophic differentiation (**b**), and inhibit terminal differentiation (**c**).

BMPs and Sox Transcription Factors

Identifying the downstream targets that mediate the ability of BMPs to maintain the chondrogenic program is an area of intense interest. Key insights have come from studies examining the relationship between BMP signaling and Sox protein expression and function. Sox proteins are Sry-related HMG box transcription factors. *Sox9* is the earliest known marker for cells committed to chondrogenesis, and its essential role as a regulator of chondrogenic differentiation has been confirmed by genetic studies. Loss of *Sox9*, or deficiency of both *L-Sox5* and *Sox6* results in loss of most skeletal elements. In *Sox9* mutants, chondrogenic condensations never form and no cartilage specific markers are expressed. Sox9 is expressed continuously in chondrocytes up to the hypertrophic stage, and is required to maintain the chondrocyte phenotype. L-sox5 and Sox6 are expressed in chondrocytes, have overlapping functions, and are required for maintenance of the chondrocyte phenotype [Bi et al., 1999; Smits et al., 2001]. Several studies have examined the relationship between BMP signaling and L-Sox5, -6, and -9 expression [Zehentner et al., 1999; Chimal-Monroy et al., 2003; Fernandez-Lloris et al., 2003] (Fig. 2). BMPs promote the expression of *Sox9* in C3H10T1/2 cultures, placing BMPs upstream of Sox9 in these cells. These studies also show that *Sox9* expression is required for BMP induced chondrogenesis, as antisense Sox9 oligonucleotides block the ability of BMPs to induce type II collagen [Zehentner et al., 1999; Fernandez-Lloris et al., 2003]. The ability of BMPs to induce *Sox* gene expression has also been investigated in vivo. Implantation of BMP2 beads near condensed cartilage leads to upregulation of Sox proteins in condensations, and Noggin beads lead to severe down-regulation of these genes. This regulation is time dependent: Sox9 is induced within an hour and L-Sox5 and -6 are induced later. This pattern of induction leaves open the possibility that BMPs directly regulate Sox9, triggering chondrogenesis, which subsequently leads to *L-Sox5* and *-6* expression. These studies show that BMPs are not sufficient to regulate Sox protein expression in limb mesenchyme. BMP beads can only induce *Sox* genes in condensed cells. In addition, when *Sox* genes are induced by TGFβ rather than BMP in mesenchyme, Noggin does not inhibit *Sox* gene expression [Chimal-Monroy et al., 2003]. These results indicate that BMP signaling alone is not sufficient to initiate *Sox9* expression prior to condensation, but that BMP signaling is required for the maintenance of *Sox9* expression in condensations. The molecular mechanisms underlying the ability of BMP signaling to maintain *Sox9* expression are unknown.

BMPs in Early Chondrocyte Differentiation

The requirement for BMPs to maintain *Sox* gene expression is consistent with the results of many studies demonstrating a continuous requirement for BMPs during chondrocyte differentiation. This aspect of BMP action has been studied extensively in ATDC5 chondrosarcoma cells. These cells can undergo several aspects of chondrogenic differentiation that occur during endochondral ossification. Treatment of ATDC5 cells with BMPs results in changes in cell morphology characteristic of chondrocytes and an upregulation of type II collagen production [Shukunami et al., 1998, 2000]. Conversely, the overexpression of a DN type I BMP receptor or treatment with Noggin reduces cartilage formation and type II collagen prodcution [Ito et al., 1999; Shukunami et al., 2000]. The clearest in vivo evidence demonstrating that continuous BMP signaling is required in chondrogenesis comes from studies in chick limbs. Precartilaginous cells in condensations do not differentiate into chondrocytes in the absence of BMP signaling [Pizette and Niswander, 2000].

BMP Action in the Growth Plate

In addition to roles in early chondrogenesis, BMPs have important functions in the growth plate at later stages. *Bmp2, -4*, and *-5* have overlapping expression patterns in the perichondrium, *Bmp7* is expressed in both the perichondrium and proliferating chondrocytes, and *Bmp6* is expressed in prehypertrophic and hypertrophic chondrocytes [Lyons et al., 1995; Pathi et al., 1999; Minina et al., 2001]. Type I BMP receptors also have both distinct and overlapping expression patterns. In addition to overlapping expression of *Bmpr1a* and *1b* in prechondrogenic condensations, *Bmpr1a* is expressed in prehypertrophic and hypertrophic chondrocytes, while *Bmp1b* is found throughout the growth plate; *Alk-2* is expressed primarily in resting and proliferating chondro-

cytes [Zou et al., 1997; Zhang et al., 2003]. As these expression patterns suggest, BMPs have functions in the growth plate at various stages of differentiation. That BMPs promote proliferation in the growth plate is well established. This was first demonstrated by the observation that *noggin* null mice have overgrown skeletal elements [Brunet et al., 1998], leading to the inference that in the absence of this antagonist, chondrocytes exhibit a proliferative response to endogenouse BMPs. In accordance, overexpression of *noggin* results in smaller growth plates and reduced proliferation, while application of BMPs increases proliferation rates and growth plate size [Pathi et al., 1999; De Luca et al., 2001; Minina et al., 2001]. In addition to the roles of BMP pathways in regulation of type II collagen expression in proliferating and columnar chondrocytes, BMPs promote terminal differentiation. In ATDC5 cells and chick chondrocytes, BMPs increase the expression of type X collagen, the major ECM marker for hypertrophic chondrocytes [Shukunami et al., 1998; Grimsrud et al., 1999; Ito et al., 1999]. This effect is direct because BMPs upregulate type X collagen promoter activity [Volk et al., 1998]. In vivo experiments have verified a role of BMP pathways in hypertrophic differentiation; exposure to Noggin reduces the zone of type X collagen expressing hypertrophic chondrocytes, indicating a requirement for BMPs to exit the cell cycle and initiate terminal differentiation. Noggin also increases the expression of osteopontin, a marker for the most differentiated cells in the growth plate in these experiments [Pathi et al., 1999; De Luca et al., 2001; Minina et al., 2001]. Thus, BMPs promote chondrocyte differentiation toward the initial hypertrophic state, but may inhibit the most terminal stages of differentiation.

BMP Receptors in Chondrogenesis

The previous studies suggest that BMPs are required to maintain the chondrocyte phenotype. This implies that BMP signaling regulates the distinct patterns of gene expression characteristic of the different populations of chondrocytes within the growth plate. Identification of the signaling pathways through which BMPs act to control distinct aspects of chondrocyte proliferation and differentiation is an area of intense interest. Although BMPs bind to BMPRIA, IB, and ALK-2, it is unclear whether these receptors elicit distinct effects during chondrogenesis. Overexpression of CA-BMPRIA or IB in chick limb buds results in identical expansions of cartilaginous elements and chondrocyte proliferation [Zou et al., 1997]. However, several lines of evidence suggest that they also have some unique functions. In the chick, *Bmpr1a* is expressed at low levels throughout limb bud mesenchyme, whereas *Bmpr1b* is expressed in precartilaginous condensations prior to type II collagen expression. The predominant expression of bmpr1b in condensations indicates that this receptor might be the primary mediator of BMP signaling during condensation. Consistently, DN-BMPRIB overexpression in limb bud micromass cultures results in a complete absence of cartilage nodule formation, while DN-BMPRIA overexpression has no effect [Zou et al., 1997]. In accordance, overexpression of DN-BMPRIB leads to inhibition of proteoglycan, type II collagen and aggrecan expression, while DN-BMPRIA leads to only a slight inhibition in chick chondrocytes [Enomoto-Iwamoto et al., 1998]. Thus, these studies suggest that while both BMPRIA and IB are capable of inducing chondrogenesis when overexpressed, BMPRIB is the major transducer of BMP signals in condensations. Whether or not BMPRIA and 1B transduce qualitatively distinct signals is unclear. The effects of overexpression of CA-receptors suggest that the signaling pathways activated by these receptors lead to similar outcomes in chondrocytes. Moreover, although DN-BMPRIB blocks chondrogenesis in chick limbs, *Bmpr1b* null mice have a mild skeletal phenotype, characterized by severe defects in phalangeal elements, but minor changes in other endochondral elements [Baur et al., 2000; Yi et al., 2000]. These results raise the possibility that there may be species-specific differences in the degree of overlapping function of BMPRIA and BMPRIB. That BMP receptors have overlapping functions in mammals is shown by the more severe phenotype of mice lacking both BMP7 and BMPRIB [Yi et al., 2000]. In these mice, a number of skeletal elements are severely reduced or absent, demonstrating that BMP7 can activate BMPRIA and/or ALK-2 in vivo, and that these receptors have overlapping functions with BMPRIB. Our ongoing analyses of mice lacking BMPRIA and IB in cartilage are consistent with broadly overlapping functions for these receptors at early stages of chondrogenesis [Yoon et al., 2003].

Evidence from a variety of studies suggests that ALK-2 has effects on chondrogenesis that are very distinct from those elicited by BMPRIA and IB. In ATDC5 cells, CA-BMPRIA or IB increases cartilage nodule formation, while CA-ALK-2 does not. In fact, overexpression of CA-ALK-2 actually delays differentiation in primary chondrocytes by inducing expression of parathyroid hormone-related protein (PTHrP), an inhibitor of hypertrophic differentiation [Zhang et al., 2003]. Moreover, when DN-BMPRIA or IB is overexpressed in ATDC5 cells, chondrogenic differentiation is blocked, while DN-ALK-2 overexpression has no effect [Fujii et al., 1999]. These results suggest that although ALK-2 can activate the same subset of BMP-specific Smads as BMPRIA and IB in many cell types in vitro, signaling through BMPR1A and 1B promotes chondrogensic differentiation whereas signaling through ALK-2 may inhibit it. Consistent with this possibility, *Alk-2* is highly expressed in the resting and proliferative zones, areas where chondrocytes need mechanisms to prevent premature differentiation [Zhang et al., 2003]. The mechanistic basis for these apparent differences is unknown. It may be qualitative; these receptors may activate different non-Smad mediated signaling pathways. The differences may also be quantitative, with distinct outcomes arising as a result of different threshold requirements for BMP signal transduction in distinct aspects of chondrocyte proliferation and differentiation. The possibility that distinct combinations of BMP receptors transduce distinct effects has also been raised. While CA-ALK-2 has either no, or an inhibitory effect on its own, it synergizes more strongly with BMPRIA and 1B to induce chondrogenesis than these latter receptors do with each other [Aoki et al., 2001]. Understanding the mechanistic basis and physiological significance of these interesting observations will require in vivo studies.

Intracelluar Signaling Pathways: Smads vs. p38

While the importance of Smad-mediated signaling in BMP-induced chondrogenesis is thoroughly established, the roles this pathway plays in early chondrogenesis are unclear, as there are conflicting results depending on the cell line examined. In C3H10T1/2 and ATDC5 cells, overexpression of BMP specific Smads does not efficiently promote cartilage formation, but the extent to which this reflects technical difficulties in overexpressing Smads is unclear. Moreover, in ATDC5 cells, overexpression of smad1, -5, or -8 in conjunction with Smad4 cannot rescue the anti-chondrogenic effects of DN-BMPRIB overexpression [Fujii et al., 1999; Ju et al., 2000]. In contrast, in the chondroprogenitor cell line MC615, overexpression of BMP smads in conjunction with Smad4 mimics the chondrogenic effects of BMP treatment. In addition, Smads 1, and 5 activate the type II collagen promoter [Hatakeyama et al., 2003].

One of the most important insights that has emerged from recent studies is that in addition to mediating effects through canonical Smad pathways, BMPs effect chondrocytes by activating p38 MAPK [Nakamura et al., 1999; Ju et al., 2000; Hatakeyama et al., 2003; Seto et al., 2004]. In ATDC5 cells, BMP treatment leads to sustained phosphorylation of p38. The ability of BMPs to promote chondrogenesis requires p38, as p38 inhibitors strongly suppress induction of type II collagen and chondrogenic differentiation without effecting cell proliferation [Nakamura et al., 1999].

The combined data from these and other studies indicates that Smad and p38 mediated BMP signaling play essential and nonoverlapping roles in chondrogenic differentiation. In vivo studies will be required to elucidate the extent to which these pathways mediate distinct aspects of chondrogenesis and the extent to which they interact.

Synergy Between BMP Signaling and the Indian Hedgehog (IHH)/Parathyroid Hormone-Related Protein (PTHrP) Pathway

The diverse functions of BMP signaling pathways in the growth plate are impacted by interactions with other signaling pathways. One important interaction is with the IHH/PTHrP pathway (Fig. 3A). Indian hedgehog (IHH) is a member of the Hedgehog family of proteins and signals by binding to the Patched/Smoothened receptor complex, leading to activation of Gli transcription factors. *Ihh* is expressed by prehypertrophic chondrocytes, and knockout of *Ihh* results in reduced chondrocyte proliferation and premature differentiation [reviewed in Kronenberg, 2003]. This phenotype arises because *Ihh* null mice do not express PTHrP in their growth plates. PTHrP is normally expressed by perichondrial cells at the ends of skeletal elements, and inhibits hypertrophic differentiation by maintaining cells in

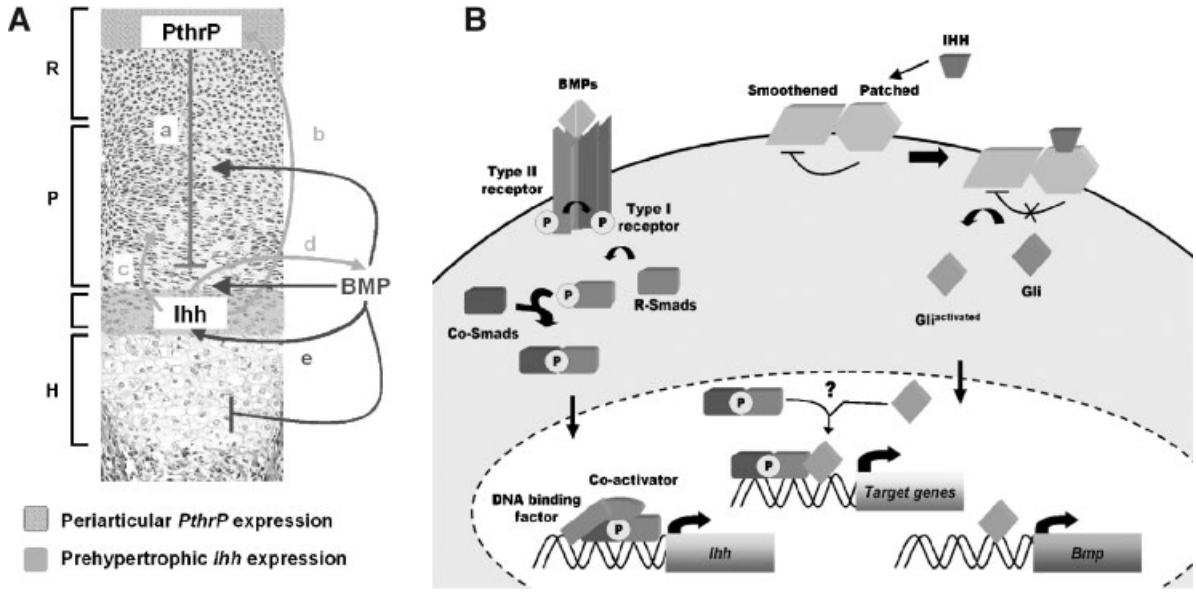

Fig. 3. Cooperation between IHH/PTHrP and BMP signaling pathways. IHH and PTHrP form a feedback loop to regulate hypertrophic differentiation in the growth plate. **A**: PTHrP inhibits hypertrophic differentiation by maintaining cells in the proliferative state (**a**). Perichondrial PTHrP expression is regulated by IHH (**b**). IHH is expressed in the perhypertrophic zone and promotes proliferation (**c**). BMPs and IHH mutually promote the expression of each other (**d**, **e**). **B**: IHH signal by binding to a receptor complex composed of Smoothened and Patched, resulting in the activation of Gli transcription factors. There is evidence from other systems demonstrating that IHH and BMPs are capable of functional synergy. Whether this synergy occurs in chondrocytes remains to be tested.

the proliferative state. This negative feedback loop between IHH and PTHrP controls the length of the proliferative zone, and thus the extent of bone growth.

BMPs interact with the IHH/PTHrP pathway by promoting *Ihh* expression. In vivo, exposure of cartilage to Noggin reduces *Ihh* expression [Pathi et al., 1999; Minina et al., 2001]. The *Ihh* promoter contains multiple Smad binding motifs and is activated by BMP treatment, demonstrating that the regulation of *Ihh* expression by BMPs is direct [Seki and Hata, 2004]. In turn, IHH maintains BMP levels, indicating the existence of a positive feedback loop between BMPs and IHH. Overexpression of *Ihh* in cartilage results in increased expression of BMPs in the perichondrium and proliferating chondrocytes [Pathi et al., 1999; Minina et al., 2001]. Furthermore, the downstream mediators of IHH signaling, Gli transcription factors, directly upregulate *Bmp4* and -7 promoter activity [Kawai and Sugiura, 2001]. Although BMP and IHH pathways participate in this feedback loop, neither pathway completely mediates the other's functions. For example, BMPs and IHH act independently of each other to regulate chondrocyte proliferation, and IHH regulates *PTHrP* expression independently of BMP signaling [Minina et al., 2001].

There is also evidence supporting synergy between BMP and IHH signaling pathways. In C3H10T1/2 cells, Sonic Hedgehog (SHH), which is functionally equivalent to IHH, increases Smad1-dependent transcriptional activity [Spinella-Jaegle et al., 2001]. Second, in a mouse limb bud cell line, although IHH cannot induce Runx2 and osteocalcin expression, treatment with a blocking antibody against IHH inhibits BMP induced expression of those two genes [Long et al., 2004]. Finally, immunoprecipitation studies show that Smad1 directly associates with truncated forms of Gli3 proteins, suggesting a possible mechanism for the synergy between IHH and BMP pathways [Liu et al., 1998] (Fig. 3B). The relevance of these synergistic interactions between BMP and IHH signaling pathways in chondrogenesis needs to be verified in vivo.

Antagonism Between BMP and FGF Signaling Pathways in the Growth Plate

Fibroblast growth factor (FGF) signaling pathways play multiple essential roles in chondrogenesis. As with BMPs, the majority of FGFs are expressed in the perichondrium. FGF receptors have distinct domains of expression, with *Fgfr1* expression in prehypertrophic and hypertrophic zones, while *Fgfr3* is expressed

by proliferating chondrocytes. In humans and mice, activating mutations in FGFR1, -2, or -3 inhibit proliferation and chondrocyte differentiation, causing many of the dwarfing chondrodysplasias observed in humans. Conversely, *Fgfr3* null mice exhibit enlarged skeletal elements due to increased rates of chondrocyte proliferation (Fig. 4A) [reviewed in Ornitz and Marie, 2002]. These effects that are essentially opposite to those elicited by BMPs, suggesting that BMP and FGF pathways act antagonistically to regulate proliferation. Although FGF signaling inhibits initial hypertrophic differentiation, limb culture studies demonstrate that FGFs promote terminal hypertrophic differentiation [Minina et al., 2002]. As described above, these effects are opposite to those elicited by BMPs. The effects of FGFs are mediated by two signaling pathways: the Janus kinase-signal transducer and activator of transcription (JAK-STAT) and the mitogen-activated protein kinase/ERK kinase 1 (MEK1) pathways [Ornitz and Marie, 2002; Murakami et al., 2004]. Several recent studies have dissected the role of each pathway in FGFs signaling during chondrocyte differentiation. Crossing *Fgfr2* overexpressing transgenic mice into a *STAT1* null background rescues the increase in apoptosis and reduction in chondrocyte proliferation observed in *Fgfr2* overexpressing mice [Sahni et al., 2001]. On the other hand, activation of the MEK1 pathway in chondrocytes recapitulates the delay in differentiation observed in mice with activating mutations in FGFR3, without altering proliferation rates [Murakami et al., 2004]. Taken together, these results suggest that the JAK-STAT signaling pathway mediates the ability of FGF signaling to inhibit chondrocyte proliferation while the MEK1 pathway is responsible for the inhibitory effect of FGFs on hypertrophic differentiation.

The functional antagonism between BMP and FGF signaling pathways is further confirmed in limb culture studies. BMP treatment rescues the phenotype of FGF treated growth plates, and FGF treatment neutralizes the effects of BMPs [Minina et al., 2002]. It is unclear how these two signaling pathways regulate one another in chondrocytes. Some evidence suggests that FGFs and BMPs can mutually regulate the expression of signaling components. However, the evidence is conflicting as to whether the regulation is positive or negative. Overexpression of activated FGFR3 inhibits *Bmp4* expression in post-natal mouse growth plates [Naski et al., 1998] but induces *Bmp4* and *Bmp7* in embryonic growth plates, where BMP treatment also induces *Fgf18* expression [Minina et al., 2002]. These conflicting results could be due age differences in the mice, as suggested by the researchers [Minina et al., 2002]. Regardless, further studies must be performed

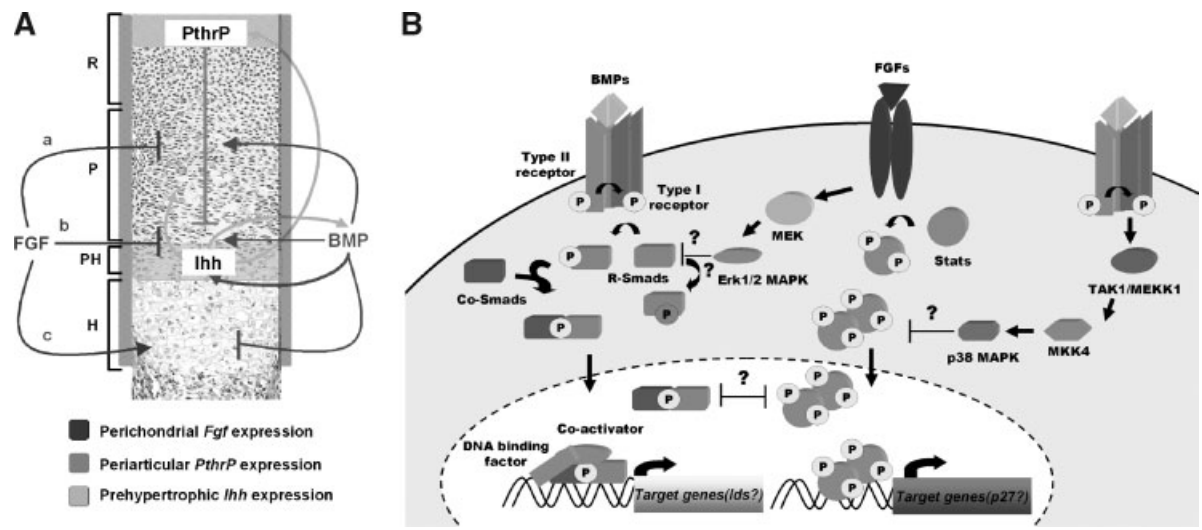

Fig. 4. Antagonism between FGF and BMP signaling pathways. **A**: FGFs are expressed in the perichondrium and have multiple functions in the growth plate. FGFs inhibit proliferation (**a**) and hypertrophic differentiation (**b**), and promote terminal differentiation (**c**), effects opposite of BMPs. **B**: FGFs signal through two primary pathways in chondrocytes: The Erk1/2 MAPK pathway and Jak/STAT pathway. It is unclear at what level(s) FGF and BMP signaling pathways intersect to antagonize one another. There is evidence that Erk1/2 MAPK can phosphorylate R-Smads and thereby inhibit them. Whether this level of regulation occurs in chondrocytes remains to be examined.

to address the issue of transcriptional regulation. It will be also important to investigate the mechanisms by which these two signaling pathways converge to antagonize one another. There is evidence from other systems demonstrating that the MEK1 pathway can phosphorylate the linker region of Smad1, subsequently inhibiting BMP signaling [Kretzschmar et al., 1999; Pera et al., 2003]. Similarly, there is evidence demonstrating that BMPs can inhibit the phosphorylation and activation of *STAT3* by an unknown mechanism [Kawamura et al., 2000] (Fig. 4B). Whether these mechanisms account for the antagonism between BMP and FGF signaling in chondrocytes needs to be examined.

Perspectives

Much of our current understanding of the mechanisms by which BMPs regulate chondrogenesis is based on in vitro studies. The use of CA receptors has revealed that chondrocytes have the potential to respond to BMP signals in multiple ways, but does not reveal the extent to which these responses are utilized in vivo. The complementary use of DN receptors confirms that BMP signaling is required for aspects of chondrogenesis, and reveals intriguing hints that different BMP receptors might transduce at least some qualitatively distinct signals. However, defining the extent to which different BMP receptors mediate distinct aspects of chondrogenesis will require in vivo genetic studies.

A major challenge for the future will be to elucidate the distinct signaling pathways, both Smad-dependent and -independent (or combinations of pathways) that mediate the effects of BMPs on distinct aspects of chondrocyte commitment, proliferation, differentiation, and survival. This effort will require a more complete understanding of the mechanisms by which BMP pathways regulate the expression of specific target genes, and the identification of additional target genes. It is likely that, as is the case for FGF signaling, distinct pathways activated by BMP receptors will mediate distinct effects on chondrocyte behavior. Unraveling these pathways and understanding how they intersect with those activated by other regulators of chondrocyte behavior (e.g., FGFs, Ihh, PTHrP, Wnts, IGFs) will be a considerable challenge, but is likely to provide insights that will enable new therapeutic approaches to cartilage repair and replacement.

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