

Regulating Bone Growth and Development with Bone Morphogenetic Proteins

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ABSTRACT: There are many gene products reported to promote osteoblast differentiation and thus increase bone formation, but only the transcription factor Runx2 and members of the bone morphogenetic protein (BMP) family of growth/differentiation factors have been shown to be absolute requirements for osteogenesis. Mice lacking the transcription factor Runx2 (also known as cbfa1) develop no bone. Similarly, osteoblast differentiation and bone formation is blocked when BMP signaling is suppressed by overexpression of noggin, a selective BMP antagonist. It is therefore not unexpected that several different mechanisms have evolved to regulate the effects of BMP-induced signaling. In this session we focus on the multiple ways in which cells can modulate BMP-induced osteogenesis and mechanisms by which BMP signaling can lead to transcriptional control of gene expression.

KEYWORDS: skeleton; bone growth; bone development; osteoblast; bone morphogenetic proteins; TGF- β

The term *bone growth* can encompass many events, including developmental establishment of skeletal elements, enlargement of bone primordia, and elongation of long bones. In this article we use the term to refer to actions leading to deposition of new bone via signaling molecules that stimulate osteoblast differentiation. The bone may be formed via endochondral or intramembranous pathways, and may be ectopic or at bony sites. The unifying characteristic is that this bone growth occurs when precursor mesenchymal cells or osteoprogenitors are stimulated with secreted molecules that are members of the TGF- β superfamily of growth and differentiation factors. Since there are many recent publications reviewing growth factor regulation of bone growth,¹⁻⁴ in this brief overview I will highlight some recent findings related to the specificity of action and signaling mechanisms used by these factors.

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The major osteoblast-stimulating factors within the TGF- β family are found among both the bone morphogenetic proteins (BMPs) and the related growth and differentiation factors (GDF). The variety and evolutionary relationships of these proteins are described by Rosen later in this volume. BMPs were named for the ability to induce ectopic bone formation, and the name implies that they are capable of inducing the osteogenic differentiation of non-bone cells.^{5,6} This is true for most of the best-studied BMPs, but not all. Furthermore, the name does not acknowledge the breadth of BMP actions. The observation that mouse development was blocked at the gastrula stage in the absence of either BMP2 or BMP4 was the first indication that BMPs are more appropriately characterized as “body morphogenetic proteins.” They are now known to specify developmental cell fate during embryogenesis and determine the dorsoventral body axis. They are involved in the formation and patterning of the nervous system, heart, gut, and kidney as well as skeletal tissues and teeth.

With the discovery that BMPs affect development of many tissue types came the recognition that BMP actions in early embryos could not be explained simply as the presence or absence of ligands that can activate BMP receptors. Instead, the body morphogenetic actions of BMPs are now viewed as mediated by the formation and maintenance of gradients. For example, the current view of neural crest induction during gastrulation is that the neural plate forms at regions of low BMP levels, while intermediate BMP signaling leads to neural crest formation and high BMP signaling results in non-neural ectoderm.⁷ One mechanism for developing such gradients is to have a BMP ligand produced and secreted at a discrete site, such that concentrations decrease with increasing distance from the source. Another mechanism is to form gradients of active ligand by gradually sequestering some of the ligand in an inactive form. Research in the past decade has revealed several secreted molecules, such as noggin, chordin, follistatin, and gremlin, which bind to BMPs, thereby inactivating them and creating a gradient of free BMP available for receptor binding. Studies of *Xenopus* development indicate that BMP antagonists derived from dorsal mesoderm diffuse in the ectoderm to create a gradient of BMP activity, establishing an intermediate level of BMP required for neural crest formation. Similarly, the low BMP levels leading to neural plate formation reflect high levels of BMP inhibitors, and zebrafish embryos lacking functional chordin have a reduced neural plate.⁷

Studies in postnatal animals indicate that the continued osteogenesis required for bone growth, bone remodeling, and bone repair in vertebrates is regulated by the availability of a subset of BMPs including BMP-2, -4, -6, -7, and -9. Like the systems operating in early embryonic development, the levels of functional BMPs can be controlled by the production of BMP-binding proteins that antagonize BMP action, such as noggin and chordin. Dr. Rosen's article reviews several additional ways in which levels of functional BMP can be regulated, including retention of the pro-region after BMP processing and

the existence of compounds that can modify BMP binding to BMP receptors. However, regardless of the mechanism for regulating BMP availability, it is questionable whether gradients of active BMP similar to that seen in early morphogenesis play a significant role in defining localizing sites of osteogenesis. Many of the systems modulating BMP-induced osteogenesis do so because BMP-activated signaling triggers negative feedback mechanisms in the target cell. For example, exposure of mesenchymal and osteoblastic cells to BMP rapidly induces expression of the secreted BMP antagonist noggin as well as Smad6 and Smad7, the intracellular inhibitors of BMP signaling. It is logical that the major systems influencing BMP activity in bone formation are primarily regulated temporally rather than spatially, consistent with the very different roles of BMPs in early development and in postnatal maintenance of the skeleton. In embryogenesis, BMPs define embryo polarity and patterning, whereas in the maintenance of the skeleton they act to recruit osteoblast precursors to a defined location in response to a specific need.

Although BMPs have been intensively studied for 15 years, it is still not clear what controls whether a specific BMP will be osteoinductive. Curiously, BMP2 and BMP4, which are absolutely required for the embryo to develop beyond the gastrula stage, also can promote postnatal osteogenesis. BMP-2 and BMP-7 are the most extensively studied for their ability to promote osteogenesis *in vivo* and *in vitro*, but recent studies suggest that BMP-6 and BMP-9 may be more effective in promoting orthotopic bone formation.^{8,9} In contrast, although BMP3 is included in the bone morphogenetic protein category on the basis of homology, it inhibits osteogenesis.¹⁰ One way to provide specificity for BMPs would be to have a variety of receptors specialized for different functions. Functional BMP receptors are heterodimers, created by combinations of three type I and three type II receptors.⁴ While multiple pairings could provide enough combinations to have specific receptors for each BMP, this does not appear to be the case, since both overexpression and knockout studies suggest that several BMPs can use one receptor pair and the absence of a receptor does not totally block signaling activated by specific BMPs.¹¹

The number of intracellular pathways activated by BMPs is relatively limited, suggesting that this will not be a source of much specificity in BMP action. The major signaling pathway downstream of BMPs is receptor activation of Smad1, Smad5, or Smad8, although there is increasing evidence that p38 mitogen-activated kinase can function as an alternative pathway.^{12,13} Activation of Smads occurs by serine phosphorylations at the C terminus, which permit binding to co-Smad4 and nuclear translocation of the Smad complex. The activated Smad complex is capable of binding to specific response elements on DNA and thus acting as a transcription factor. However, its DNA-binding affinity is relatively weak, and effective Smad transcriptional regulation appears to rely on the Smad complex dimerizing with other DNA-binding proteins (co-activators or co-modulators), which have higher affinity for their response element sequences on DNA. BMP-activated Smads,

TGF- β -activated Smads, and their common co-Smad, Smad4, all have DNA-binding regions that can recognize similar response elements. Initial studies examining the DNA sequences needed for Smad-binding identified a GTCT motif, a GCAT motif, and a GCCG motif. These seemed to work best as multiple copies and none of them appeared specific for individual activated Smads. Karaulanov *et al.*¹⁴ have recently brought some clarity to the question of Smad-binding sites, characterizing the promotor regions of several BMP-responsive genes and demonstrating a pattern in which the sequence TGGCGCC (Bre7 motif) is located a short distance upstream of one or more SBE (Smad-binding element) motifs containing GTCTG. A more extensive genome scan for vertebrate BMP response elements, combined with functional promotor assays, has identified several dozen genes that are candidates for regulation by activated Smads.¹⁵

A central unanswered question in the field of BMP-induced osteogenesis is how BMP-activated signaling results in the expression of an osteoblast phenotype. Because BMP treatment (or overexpression of activated BMP receptors) ultimately results in elevated alkaline phosphatase expression as well as increased production of bone matrix proteins, it is frequently assumed that BMP-activated Smads directly regulate the expression of these genes. One popular theory is that BMP-activated Smads bind to activated Runx2 (*cbfa1*), the transcription factor required for bone formation, thereby forming a transcriptional complex that regulates the expression of key osteoblast genes. However, there is little evidence for this. In his presentation, Dr. Cao discusses one mechanism by which BMP-activated Smads might regulate osteoblast gene expression without DNA binding; activated Smad relieves repression of the osteopontin gene by binding to the repressor *Hoxc8* and removing it from the osteopontin promoter.¹⁶ An examination of genes identified as having Smad-responsive promoters, based on phylogenetic analysis combined with functional promoter assays, identified over 100 putative BMP target genes. None of them were osteoblast-specific genes, but a very high proportion of them were transcriptional regulators.¹⁵

It therefore seems increasingly likely that many reports showing BMP-mediated increases in osteoblast gene expression actually involve an indirect mechanism, in which the initial rapid downstream effect of BMP is the binding of activated Smads to the promoter of a gene encoding a transcriptional regulator or an activator of signaling intermediates. In this scenario, proteins coded by these genes would subsequently affect the expression of osteoblast genes such as alkaline phosphatase, osteopontin, and bone sialoprotein. From the viewpoint of achieving bone formation, this is a mechanistic distinction that might suggest additional therapies for modulating osteogenesis. From a semantic perspective, it would argue that bone morphogenetic proteins not only have a developmental role extending far beyond what the name would suggest, but they also have no direct effect on genes associated with an osteoblast phenotype.

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