

## Commentary

# Interplay between bone morphogenetic proteins and cognate binding proteins in bone and cartilage development: noggin, chordin and DAN

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### Abstract

This commentary is a concise discussion of the interactions between bone morphogenetic proteins (BMPs) and their binding proteins in bone and cartilage morphogenesis. BMPs are a family of growth and differentiation factors, and they act on mesenchymal cells to induce cartilage and bone differentiation in concentration-dependent thresholds. The BMP–BMP receptor binding leads to a cascade of signaling and transcription of BMP response genes. BMP binding proteins, noggin, chordin and DAN, act as antagonists and determine the bioavailability of BMPs for binding to cognate receptors to elicit the biological response. Noggin null mice with unrestricted action of BMPs exhibit defects in joint morphogenesis. BMPs and their binding proteins may reciprocally regulate the dynamic topography of joints, muscle, tendons and ligaments during morphogenesis of the skeleton. In addition, BMP actions may be potentiated by twisted gastrulation. BMPs and their binding proteins may play a critical role in regeneration of cartilage in osteoarthritis.

**Keywords:** BMP, BMP receptors, extracellular matrix, Smads

### Introduction

The sequelae of damage and degradation of articular cartilage is well known in osteoarthritis. Repair and regeneration of articular cartilage in experimental animals is initiated when the subchondral bone is penetrated, as in full thickness defects. On the other hand, there is no repair in partial thickness defects with the defect confined to cartilage only. Subchondral bone with associated matrix and hematopoietic stromal cells may thus play a role in cartilage repair. The difference in regenerative potential between bone and cartilage is immense. The fundamental differences between cartilage and bone in inherent repair potential may be due to differences in concentration of endogenous growth and

morphogenetic factors and their antagonists. The demineralized bone matrix is a repository of BMPs and this might explain, in part, the repair of articular cartilage in full thickness defects. The aim of this commentary is to discuss the interplay between the evolving family of BMPs and cognate BMP binding proteins in bone and cartilage development. In arthritis, with attendant cartilage damage, there is a derangement of the morphogenetic signals. It is thus possible to harness the recent progress in developmental biology of morphogens to design rational therapeutic approaches to cartilage regeneration. Regeneration is, after all, a recapitulation of embryonic development and morphogenesis, and includes redeployment of morphogens in regeneration.

## Bone morphogenetic proteins

BMPs are a family of growth and differentiation factors [1,2]. BMPs are pleiotropic morphogens, and they induce new cartilage and bone formation in ectopic sites by a developmental sequence that mimics limb development and morphogenesis. BMPs have chemotactic, mitogenic and differentiation-inducing properties. The actions of BMPs are concentration dependent, and are based on thresholds. BMPs are dimeric proteins with a single inter-chain disulfide bond. The dimeric conformation is an absolute requirement for the biological action and interaction with receptors.

BMPs were initially identified as bioactive molecules in the demineralized bone matrix responsible for cartilage and bone differentiation [1,2]. BMPs regulate the lineage, pattern and differentiation of bone, and over 15 have been cloned and expressed in humans and mice. Genetic evidence points to actions of BMPs in tissues beyond bone. Knockout of BMP 2 and BMP 4 results in defects in mesoderm formation. Mutations in BMP 5 in mice are responsible for the short ear phenotype. BMP 7 null mice exhibit defects in eye, kidney and skeleton [2]. BMP 4 stimulates chondrogenesis in limb bud mesenchymal cells [3] and maintains articular cartilage phenotype [4].

## Cartilage-derived morphogenetic proteins

Luyten and coworkers identified cartilage-derived morphogenetic protein-1 (CDMP-1) in articular cartilage [5] and found it identical to growth/differentiation factor 5. The incisive work of Lee and coworkers cloned growth/differentiation factors 5, 6, and 7, with mutations in growth/differentiation factor 5 identified in mouse brachypodism [6]. It is noteworthy that mutations in human CDMP-1 were identified in patients with Hunter-Thompson type chondrodysplasia, type C Brachydactyly and Grebe type chondrodysplasia with severe limb shortening and impaired morphogenesis [7,8]. BMPs and CDMPs are thus critical for bone and cartilage morphogenesis and beyond. BMPs should perhaps be called body morphogenetic proteins, which would take into account the wide-ranging actions of BMPs and obviate the need for tinkering with terminology.

## BMP receptors

There are seven canonical conserved cysteines in the mature monomer, six of which are involved in intrachain disulfide bonds. The seventh is key for the interchain disulfide bond. The BMP monomer forms two finger-like projections from the palm of the hand. BMPs interact with specific receptors. Recombinant human BMP 4 and BMP 7 bind to two distinct type I BMP receptors, BMPR-IA and BMPR-IB, and BMP 7 also appears to bind to activin-type I receptor. BMPs also bind to type II BMP receptors, with both BMP receptors I and II being serine/threonine kinases. A gene from *Caenorhabditis elegans*, *daf 4*, binds to recombinant human BMP4. Expression of *daf 4* inhibits

dauer larva formation. Three genes, *Sma 2*, *Sma 3* and *Sma 4*, were identified in *daf 4* signaling in *C. elegans*. Systematic genetic screens in *Drosophila melanogaster* identified a gene mother against decapentaplegic (*Mad*). There was sequence homology between *Sma 2*, *Sma 3*, and *Sma 4* in *C. elegans* and *Mad* in *Drosophila*, and the scientists have fused the terms *Sma* and *Mad* to Smad in mammals. There are three classes of Smads. The receptor-regulated R-Smad 1, R-Smad 5 and R-Smad 8 are phosphorylated by BMP receptor kinases [9]. X-Ray crystallography has revealed the trimeric nature of Smads. The R-Smads interact with a common signaling partner Co-Smad, Smad 4. The multimeric protein complex of Smad 1/5/8 and Smad 4 is translocated into the nucleus and initiates transcription of BMP response genes. The type I receptor kinase catalyzed phosphorylation of Smad 1 and Smad 5 is inhibited by inhibitory Smads, Smad 6 and Smad 7. These inhibitory Smads are normally resident in the nucleus, act as a homeostatic relay upon BMP stimulation of cells and are translocated into the cytosol to inhibit type I BMP receptor kinase catalyzed phosphorylation of Smad 1/5. This intricate signaling is dependent on the bioavailability of BMPs at steady state to the cognate receptors. BMP levels and interaction with receptors is dependent on binding to the extracellular matrix and BMP binding proteins (Fig. 1). BMPs bind to extracellular matrix components and thus the availability of BMP for receptor binding is restricted [10]. The extracellular matrix may potentiate the biological actions of BMPs [10].

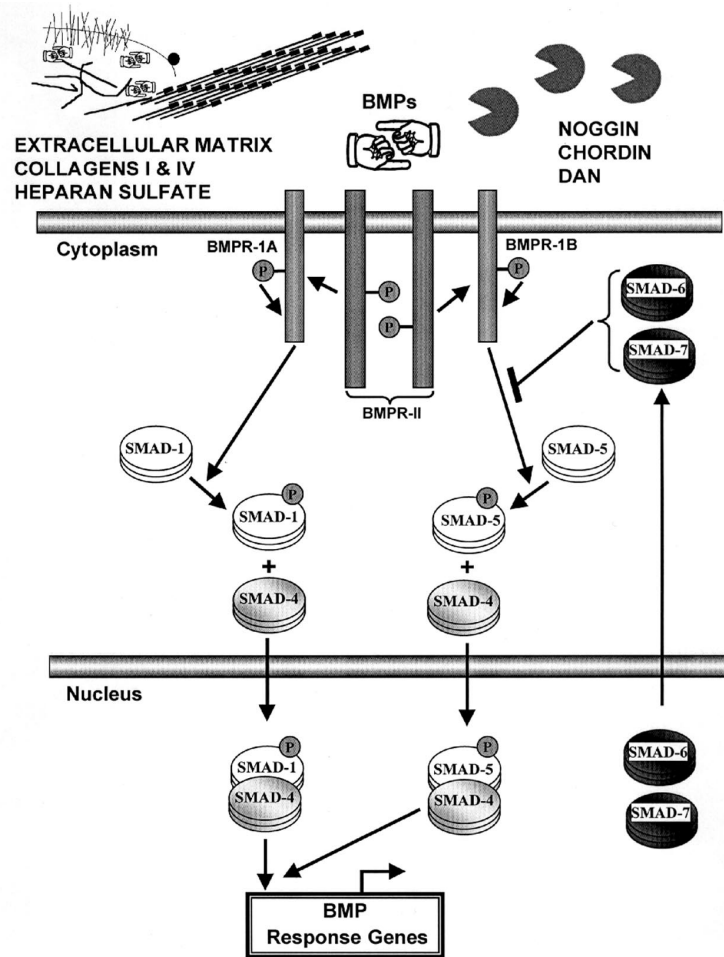
## BMP binding proteins as antagonists

### Noggin

During the course of a search for the elusive neural inducer, noggin was isolated from *Xenopus* based on rescue of dorsal development in ultraviolet-induced ventralized embryos [11]. Injection of the putative cloned RNA into embryos resulted in large heads, hence the name noggin. Noggin is produced by the Spemann organizer and antagonizes the action of BMPs, induces neural tissue and dorsalizes ventral mesoderm. Noggin binds to BMP 2 and BMP 4 with high affinity and blocks interaction with BMP receptor [12]. The bioavailability receptor is thus modulated by BMP antagonist noggin.

What has noggin to do with arthritis and articular cartilage? The precise boundary between muscle and cartilage with the interspersed perichondrium, the tendon/ligament interface, requires precisely regulated boundary conditions during joint morphogenesis. One can envisage, given the role of dominant morphogens such as BMPs and CDMPs, BMP/CDMP binding proteins (antagonists) to play a role in defining boundaries. Experimental evidence has in fact been adduced to precisely demonstrate this, using noggin null mice generated by homologous recombination [13]. Regulated cell death and joint cavitation follow mesenchymal cell condensation in regions of presumptive joint

Figure 1



BMP receptors and signaling cascades. BMPs are dimeric ligands with a cysteine knot in each monomer fold. Each monomer has two  $\beta$  sheets (represented as two pointing fingers). These fingers in the functional dimer are oriented in opposite directions. BMPs interact with both type I and type II BMP receptors (BMPR-I and BMPR-II). The exact stoichiometry of the receptor complex is currently being elucidated. BMPR-II phosphorylates the glycine-serine (GS) domain of BMPR-I. The collaboration between type I and type II receptors forms the signal transducing complex. The BMP type I receptor kinase complex phosphorylates the trimeric signaling substrate Smad 1 or Smad 5. This phosphorylation is inhibited and modulated by inhibitory Smad 6 and Smad 7. Phosphorylated Smad 1 or Smad 5 interacts with Smad 4 (functional partner) and enters the nucleus to activate the transcriptional machinery for early BMP response genes. A novel Smad interacting protein may interact and modulate the binding of heteromeric Smad 1/Smad 4 complexes to the DNA. The bioavailability of BMP for interaction with BMP receptors is determined by binding to extracellular matrix components such as heparan sulfate and collagen IV. The BMP antagonists noggin, chordin and DAN can also bind with high affinity to BMP and prevent interaction with receptors. There is thus a very intricate regulation of BMP biological actions.

morphogenesis, and CDMP-1 has been implicated in this process [13]. It is possible in mice lacking noggin that the unfettered actions of BMPs/CDMPs may lead to impaired joint formation. Defects in vertebrae, ribs and limbs were observed in homozygous mutants; heterozygous mice appeared normal. The limbs are shorter in the mutant, the joints were not demarcated and fusion of the joints was commonplace. Optimal expression of BMPs/CDMP is thus needed for the normal joint morphogenesis. Future investigations with conditional knockouts of noggin in mice will shed more light on the role of noggin in joint morphogenesis. BMP 2 induces the expression of noggin in osteoblasts

[14] and bone marrow cells [15], implying regulated expression of the BMP antagonist, perhaps to downregulate the agonist response to BMP 2. This is akin to a molecular thermostat to maintain skeletal homeostasis.

#### Chordin

During the course of investigation on *Xenopus* pattern formation, chordin was identified as BMP 4 binding protein [16]. Chordin has a homolog in *Drosophila*, short gastrulation (*sog*), known to bind to decapentaplegic, a BMP 2/4 homolog. These BMP antagonists have thus been conserved for several million years. Chordin binds to BMP 2

and BMP 4, and is further regulated by a metalloprotease BMP 1 and tolloid and *Xenopus* xolloid [17–19]. Antagonists such as chordin thus govern the intricate pattern formation by morphogens such as BMP, and in turn are proteolytically inactivated by a metalloprotease BMP 1, related to the astacin family. BMP 1 was originally identified in osteogenic extracts derived from bovine bone as it copurified with bonafide bone morphogenetic proteins 2–7 [1]. BMP 1 also functions as a procollagen c-protease. In retrospect, the term BMP-1 is a misnomer, as this BMP does not induce bone morphogenesis.

## DAN

The DAN gene was isolated as a candidate tumor suppressor gene in a differential hybridization screen [20]. Secreted DAN suppressed DNA synthesis in transformed cells. The head inducer gene *Cereberus* codes for a secreted protein and can induce heads in *Xenopus* embryos, and it is related to DAN in the cysteine rich domain [21]. Gremlin is a *Xenopus* homolog related to DAN that inhibits BMP 2 action and was identified by screening an ovarian cDNA library for activities inducing the secondary axis [22]. DAN family members are thus newly identified BMP antagonists [23–25]. It is not yet clear if they play a role in articular cartilage and in arthritis.

## BMP potentiating agonist: twisted gastrulation

One of the emerging themes in developmental biology is the regulation of pattern formation and morphogenesis by sets of genes. The anterior–posterior axis, for example, is governed by *hox* genes. The dorsal–ventral axis is the result of the activity and expression pattern of bone morphogenetic proteins, BMP antagonist chordin and metalloprotease xolloid. Recent work in *Xenopus* has identified a gene ‘twisted gastrulation’ (xTsg), as an agonist of BMP actions [26]. In vertebrates, the dorsal–ventral pattern is regulated by a gradient of BMP activity. Although BMPs are expressed uniformly, the expression of BMP antagonists such as chordin generates the dorsal–ventral gradients. Further control is exerted by xolloid, a zinc-dependent metalloprotease. The xTsg binds to BMPs with a dissociation constant in the low nanomolar range. It is noteworthy that microinjection of xTsg results in potentiation of BMP signaling, leading to ventralization of *Xenopus* embryo. Twisted gastrulation is thus an agonist of BMP signaling. xTsg may thus activate the inactive BMP–chordin cysteine rich domain complex by releasing the bioactive BMPs. It is important to bear in mind, given BMPs bind to extracellular matrix [10,27] components such as collagens, heparan sulfate and heparin, additional regulation is inevitable, and is a question of time and space!

## Conclusion

The recent advances in the developmental biology of BMP binding proteins will allow a rational approach to the chal-

lenges of articular cartilage repair by morphogens. It is remarkable how Nature zealously regulates the intricate interplay between BMPs, BMP receptors, extracellular matrix, BMP antagonists such as noggin, chordin and the DAN family, and BMP potentiating twisted gastrulation.

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