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Systems control of BMP morphogen flow in vertebrate embryos

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Abstract

Embryonic morphogenetic programs coordinate cell behavior to ensure robust pattern formation. Having identified components of those programs by molecular genetics, developmental biology is now borrowing concepts and tools from systems biology to decode their regulatory logic. Dorsal-ventral (D-V) patterning of the frog gastrula by Bone Morphogenetic Proteins (BMPs) is one of the best studied examples of a self-regulating embryonic patterning system. Embryological analyses and mathematical modeling are revealing that the BMP activity gradient is maintained by a directed flow of BMP ligands towards the ventral side. Pattern robustness is ensured through feedback control of the levels of extracellular BMP pathway modulators that adjust the flow to the dimensions of the embryonic field.

Introduction

Developmental biology aims at reverse engineering the genetic program that converts a single cell into a fully functional adult animal. Cell-cell communication over long distances is an essential component of the program that organizes embryonic tissues. The BMP pathway is the main signaling pathway involved in the D-V patterning of the gastrula embryonic field, which consists of about 10,000 cells in embryos of the frog *Xenopus*. Most of its molecular components have been conserved throughout the evolution of metazoans, but despite years of genetic and biochemical studies, we are only now starting to unravel the built-in complexity of the pathway. The main focus of this review is the extracellular regulation of D-V patterning, which has emerged as a paradigm in vertebrates for the study of positional information and patterning. Its role is to reliably provide positional information along the D-V axis to instruct the histotypic differentiation of the three germ layers. For example, in the ectoderm low levels of BMP signaling give rise to central nervous system, intermediate levels to neural crest, and high levels to epidermal differentiation, while in the mesoderm the BMP gradient induces notochord, somite, lateral plate and blood island differentiation. We will first briefly review the biochemistry of the system (detailed reviews are available in [1,2*,3]). Second, we address the current understanding of the organization of the genetic network that controls D-V patterning. Finally, we examine how systems biology modeling helps understand its logic.

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Components of the BMP pathway

BMP proteins are extracellular ligands of the TGF β superfamily of growth factors. Several BMPs are expressed during *Xenopus* gastrulation, including BMP2/4/7 and ADMP (Anti Dorsalizing Morphogenetic Protein). BMP signaling is triggered by the binding of secreted BMP homo or heterodimers to two type I and type II BMP receptors. The activated receptor complex phosphorylates two serines in the C-terminus of transcription factors of the Smad family (Figure 1), resulting in their accumulation in the nucleus and activation of target genes [3]. At the extracellular level, multiple evolutionarily conserved nodes of regulation allow embryos to self-regulate, adjusting to changes in BMP gradient. Regulation occurs at three important steps: binding of BMPs to their receptors, cleavage of the BMP antagonist Chordin (Chd), and diffusion of BMP ligands in the extracellular space.

Binding of BMPs to their type I and II heterotetrameric plasma membrane receptors is highly regulated by diffusible and cell-surface bound antagonists (Figure 1). Diffusible antagonists include Chordin, Noggin, Follistatin and Chordin-like proteins [4–9]. They were originally identified as proteins secreted by the dorsal Spemann organizer able to induce twinned axes when expressed on the ventral side of *Xenopus* embryos. Chordin [10,11] and Noggin [12] physically prevent BMPs from binding to their cognate receptors (step 1 in Figure 1). In the case of Chordin, the stability of the Chd/BMP complex is greatly increased by a third protein, Twisted Gastrulation (Tsg) [13–15] (step 2 in Figure 1). The transmembrane antagonist BAMBI (BMP and Activin Membrane-bound Inhibitor) is a type I BMP truncated pseudo-receptor that associates with type II receptors and BMP ligands to form inactive signaling complexes [16] (step 8 in Figure 1).

An interesting case is that of the secreted BMP modulator Crossveinless 2 (CV2). CV2 contains Chordin-like Cysteine-Rich (CR) domains that serve as BMP-binding modules [17,18*,19*] (steps 3 and 6 in Figure 1). Although it is a secreted protein, the diffusion of CV2 is restricted to the cells that produce it and a few additional nearby cells, because it binds tightly to cell-surface Heparan Sulfate Proteoglycans (HSPG) such as glypicans [18*] (Figure 1). CV2 displays both pro- and anti-BMP activities depending on its concentration [18*]. At low concentrations, it can form a transient ternary complex with type I BMP receptors and BMP ligands that promotes the formation of signaling receptor complexes (step 6 in Figure 1). At high concentrations, CV2 sequesters BMP ligands and drives their endocytosis and degradation [20*]. Remarkably, CV2 in the ventral side of the embryo is able to bind with high affinity to Chd/BMP complexes diffusing from the dorsal side [21**]. By acting as a sink concentrating Chd/BMP, CV2 promotes peak BMP signaling on the ventral side of the embryo by facilitating the release of BMPs from Chordin [21**,22] (steps 5 and 7 in Figure 1).

The second key node of regulation is the cleavage of Chordin by Tolloid Zinc metalloproteinases, which triggers the release of active BMPs from the Chd/BMP complex [23,24] (steps 4 and 5 in Figure 1). Thus, inhibition of BMP by Chordin is reversed by a proteolytic step that constitutes the rate-limiting reaction in D-V patterning [25*]. Consequently, the activity of this protease is exquisitely regulated. Chordin cleavage is enhanced by Ont1, a dorsal secreted protein of the Olfactomedin family, which provides a scaffold that brings the Chordin substrate and the Tolloid enzyme together, facilitating proteolysis [26**] (step 3 in Figure 1). There are three Tolloid enzymes in *Xenopus*, and their activity is negatively regulated by the secreted Frizzled-related proteins Sizzled and Crescent which act as competitive inhibitors of Chordin cleavage [27–29], and by BMPs through a non-competitive inhibition mechanism [30**] (see boxes in Figure 1).

Finally, regulation of BMP transport is crucial to its role as a morphogen. Different modes of morphogen transport have been proposed, including diffusion, transcytosis and cytonemes [31–33]. So far, only diffusion has been shown to occur in vertebrates [34]. In *Drosophila*, where this question has been more thoroughly investigated, extracellular HSPGs [34,35], Collagen IV [36^{**}], and Pentagone/Smoc [37] have been implicated in the regulation of BMP transport. Collagen IV, a component of the basement membrane, offers a particularly interesting example of the molecular control of directional diffusion. Collagen IV binds to the *Drosophila* BMP4 and Chordin homologs, sequestering them in the extracellular matrix. Twisted gastrulation (a protein that binds both to BMP and Chordin/Short gastrulation [13]) serves as a solubilizing agent that releases Chd/BMP complexes from the collagen IV matrix of the perivitelline space, allowing their diffusion [36^{**}]. In *Xenopus*, it has been found that BMP4 has a shorter range of diffusion than that of other TGF β ligands [38], because the N-terminus of the mature peptide binds heparan sulfate in HSPGs via basic residues [39]. HSPGs are important for the transport of another TGF β morphogen, Nodal [40^{*}], and regulate BMP signaling during D-V patterning [41–43]. Finally, HSPGs and Collagen IV have also been shown to enhance the binding of BMPs to their plasma membrane receptors [36^{**},44].

Organizing a self-regulating gradient

Experimental studies on genes regulating the BMP pathway in *Xenopus* have uncovered a biochemical pathway consisting of extracellular proteins, which explains how gradients of BMP activity arise and are robustly maintained in embryonic tissues. In a pleasing convergence of independent lines of evidence, extensive genetic screens in the zebrafish *Danio rerio* uncovered most of the same genes as mutations specifically affecting embryonic D-V patterning [41,43]. Early work in *Xenopus* showed that BMP4 and BMP7 were expressed on the ventral side of the gastrula (the ventral center) [4], while the BMP antagonists Chordin and Noggin were expressed in the dorsal center and behaved as morphogens that could act over long distances to pattern the embryo [10,45]. These findings suggested that a D-V gradient of BMP activity could be generated simply by these two antagonistic activities generated at opposite poles of the embryo.

As shown in Figure 2, these factors proved to provide only the core of the D-V biochemical network, as many other genes co-expressed in both centers were subsequently uncovered [46]. The two centers are under opposite transcriptional control [47,48], as ventral genes are transcribed when BMP signaling is high and dorsal genes when it is low (indicated by blue arrows in Figure 2). Unexpectedly, several of these proteins proved to be feedback inhibitors of the activity of the center they were expressed in, promoting the activity of the opposite center (Figure 2). For example, low BMP signals cause *Ont1* expression in the dorsal center, enhancing Chordin degradation [26^{**}], as well as transcriptional activation of two BMP proteins, BMP2 and ADMP. This dampens excessive activation of dorsal center genes, and promotes the expression of ventral center genes at a distance by increasing BMP signaling [47,48]. The ventral center expresses two anti-BMPs, BAMBI and CV2, as well as a competitive inhibitor of Chordin cleavage, Sizzled (Figure 2). For each action of the dorsal side there is a reaction in the ventral center. These negative feedback loops and positive cross-regulations are required for the precise adjustment of the BMP activity gradient, balancing the strength of each center [27,47]. This ensures that each embryo achieves a perfect allocation of tissue types, adjusting to environmental or experimental perturbations [46,47]. One of the best examples is when experimental bisection of a *Xenopus* blastula results in identical twins of perfect proportions; this requires extensive scaling of the size of the self-regulating morphogenetic field [48].

Communication between the two opposing embryonic centers is enhanced by a “sink” mechanism. Early evidence in *Drosophila* demonstrated that a Tolloid-generated Chordin gradient could produce a directional flow of BMP ligands toward higher BMP concentrations to generate peak levels of signaling [49,50,51]. It was later found in the frog gastrula that the ventrally expressed protein CV2 could enhance this flow by binding to Chd/BMP complexes with high affinity [21**], inducing release of active BMPs from the complex after cleavage by Tolloid [23]. This generates a molecular sink driving Chd/BMP flow towards the ventral center. The essential role of CV2 in regulating the flow of Chordin over long distances was demonstrated genetically in the mouse vertebral morphogenetic field [52]. CV2 is normally expressed in the developing vertebral body, in which Chordin protein secreted by the intervertebral disc also accumulates. However, in CV2 mutant mice the relocalization of Chordin to vertebral bodies cannot take place, as shown in Figure 3.

Experimental studies in *Xenopus* have provided estimates of the *in vivo* concentrations of many extracellular factors of the BMP pathway and the chemical reaction rates of their interactions [27,30**]. Together with functional analyses of the expression domains and transcriptional regulation of these BMP regulators, this has paved the way for computational modeling of the D-V patterning network.

Insights from modeling the BMP gradient

Mathematical and computational modeling has provided insights into the function of each component and the global behavior of the system. Intuition alone is insufficient to directly understand how such a complex network with its many negative and positive feedback loops (Figure 2) generates robust and reproducible pattern. Mathematical modeling of biological gradients was pioneered by Turing [53**], who coined the term “Morphogen” in 1952, and extended by Meinhardt [54,55]. They introduced partial differential equations as mathematical tools to model systems of diffusing morphogens through elaborations of the following basic equation:

$$\frac{\partial C}{\partial t} = D \cdot \nabla^2 C + \text{reactions}(C)$$

Variations in morphogen concentration in the embryo over time ($\partial C/\partial t$, first derivative with respect to time) are driven by Fick's law of diffusion ($D \cdot \Delta^2 C$, where D indicates diffusion rate and $\Delta^2 C$ the second derivative of morphogen concentration with respect to space) and the chemical “reactions” that lead to the formation/degradation of the morphogen species (C). These reaction-diffusion equations have shown that an activator and inhibitor pair (such as ADMP and Chordin) diffusing from the same source can generate stable patterns, provided that the inhibitor diffuses faster [54,55].

With knowledge of the biochemical affinities of the system, it has become possible to use these equations to conduct extensive quantitative simulations. Modeling predicted that a requirement for robust D-V patterning was the existence of a Chordin-driven flow of BMPs toward high concentrations of that same morphogen, both in *Drosophila* [49] and in *Xenopus* [56**]. The flow of BMP ligands towards the ventral side can be visualized indirectly in BMP-depleted *Xenopus* embryos, in which a wild-type organizer graft expressing BMP ligands (ADMP and BMP2) can restore BMP-dependent epidermal differentiation, but only at a long distance from the BMP source where they are released from inactive complexes by the action of Tolloid, as shown in Figure 4. The flow of dorsal BMPs toward higher concentrations seems to contradict Fick's law of diffusion, but the maintenance of a steady Chordin concentration gradient through its degradation by the protease Tolloid provides the chemical work that drives this facilitated diffusion. These

mathematical predictions were verified experimentally in the *Drosophila* and *Xenopus* systems [49,56**].

Computational analyses have also confirmed the importance of feedback and cross-regulatory loops in ensuring the robustness of the gradient [55–57]. The study by Ben-Zvi et al. [56**] showed that the function of ADMP is to maintain a low but steady level of BMP signaling in the dorsal side and to promote at a distance peak levels of BMP signaling in the ventral center, in agreement with experimental embryology studies [47]. In addition, the ventral feedback regulator BAMBI helps cells to reliably sense a broad range of BMP concentration by linearizing the response to BMP signaling, avoiding immediate saturation and reducing variability in D-V patterning [58**]. Computational modeling also revealed that while the competitive inhibitor Sizzled and the non-competitive inhibitor BMP are feedback inhibitors of the activity of Tolloid in the ventral center, a main inhibitor of activity of this protease on the dorsal side is its own substrate, Chordin, which is produced very abundantly [30**].

Embryos are increasingly recognized as systems that need to meet multiple and often contradictory performance objectives in order to develop into functional and well-proportioned animals, bearing parallels with artifacts designed by engineers [59]. Therefore, concepts developed for the control of engineered systems are becoming increasingly useful in understanding the mechanics of developmental networks. Indeed, Ben-Zvi and Barkai [60] have shown that the D-V network is topologically analogous to an error-correcting integral-feedback controller that adjusts morphogen activity to match the size of the embryo.

Perspectives

We have come a long way since the discovery of the embryonic dorsal organizer by Hans Spemann and Hilde Mangold in 1924 [61]. Deciphering the molecular blueprint of self-regulating morphogenetic fields is now possible. Studies in *Xenopus* have revealed the biochemical interactions of many components of a novel extracellular pathway of BMP signaling regulation and discovered the principles that explain how they generate a self-regulating D-V morphogen gradient. An area still underexplored in the vertebrates is the role of proteoglycans and the extracellular matrix in the control of morphogen diffusion, which has already been more extensively studied in *Drosophila* [34–37]. Moreover, to gain further understanding of the vertebrate D-V pathway we need to go beyond biochemistry and genetics and measure the localisation of its proteins as has been done in *Drosophila*, where it has enabled the elaboration of detailed and testable quantitative models of patterning [62,63].

Finally, our understanding of how the Chordin/BMP network is integrated both at the intracellular and extracellular level with other signaling pathways, transcriptional programs, and morphogenetic movements that specify positional information in the embryo is still a very active field of investigation [64]. Beyond D-V cell differentiation, the BMP pathway also plays essential roles in the regulation of many other organ-forming fields [48,52*]. It remains to be tested how the knowledge acquired from *Xenopus* dorso-ventral patterning translates to these systems.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Plouhinec JL, De Robertis EM. Systems Biology of the Self-regulating Morphogenetic Gradient of the *Xenopus* Gastrula. *Cold Spring Harbor Perspect Biol.* 2009; 1 :a001701.
- 2. Umulis D, O'Connor MB, Blair SS. The extracellular regulation of bone morphogenetic protein signaling. *Development.* 2009; 136:3715–3728. Excellent review providing an in-depth overview of the BMP pathway in *Drosophila*, and explaining its biochemistry and mathematical modeling. [PubMed: 19855014]
3. Wu MY, Hill CS. Tgf-beta superfamily signaling in embryonic development and homeostasis. *Dev Cell.* 2009; 16:329–343. [PubMed: 19289080]
4. De Robertis EM, Kuroda H. Dorsal-Ventral Patterning and Neural Induction in *Xenopus* Embryos. *Annu Rev Cell Dev Biol.* 2004; 20:285–308. [PubMed: 15473842]
5. Smith WC, Harland RM. Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in *Xenopus* embryos. *Cell.* 1992; 70:829–840. [PubMed: 1339313]
6. Hemmati-Brivanlou A, Kelly OG, Melton DA. Follistatin, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity. *Cell.* 1994; 77:283–295. [PubMed: 8168135]
7. Sasai Y, Lu B, Steinbeisser H, Geissert D, Gont LK, De Robertis EM. *Xenopus* chordin: a novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell.* 1994; 79 :779–790. [PubMed: 8001117]
8. Nakayama N, Han CE, Scully S, Nishinakamura R, He C, Zeni L, Yamane H, Chang D, Yu D, Yokota T, et al. A novel chordin-like protein inhibitor for bone morphogenetic proteins expressed preferentially in mesenchymal cell lineages. *Dev Biol.* 2001; 232:372–387. [PubMed: 11401399]
9. Nakayama N, Han CY, Cam L, Lee JI, Pretorius J, Fisher S, Rosenfeld R, Scully S, Nishinakamura R, Duryea D, et al. A novel chordin-like BMP inhibitor, CHL2, expressed preferentially in chondrocytes of developing cartilage and osteoarthritic joint cartilage. *Development.* 2004; 131:229–240. [PubMed: 14660436]
10. Sasai Y, Lu B, Steinbeisser H, De Robertis EM. Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in *Xenopus*. *Nature.* 1995; 377:757. [PubMed: 7477270]
11. Piccolo S, Sasai Y, Lu B, De Robertis EM. Dorsal-ventral patterning in *Xenopus*: inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell.* 1996; 86:589–598. [PubMed: 8752213]
12. Zimmerman LB, De Jesus-Escobar JM, Harland RM. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell.* 1996; 86:599–606. [PubMed: 8752214]
13. Oelgeschläger M, Larrain J, Geissert D, De Robertis EM. The evolutionarily conserved BMP-binding protein Twisted gastrulation promotes BMP signalling. *Nature.* 2000; 405:757–763. [PubMed: 10866189]
14. Little SC, Mullins MC. Twisted gastrulation promotes BMP signaling in zebrafish dorsal-ventral axial patterning. *Development.* 2004; 131:5825–5835. [PubMed: 15525664]
15. Xie J, Fisher S. Twisted gastrulation enhances BMP signaling through chordin dependent and independent mechanisms. *Development.* 2005; 132:383–391. [PubMed: 15604098]
16. Onichtchouk D, Chen YG, Dosch R, Gawantka V, Delius H, Massague J, Niehrs C. Silencing of TGF-beta signalling by the pseudoreceptor BAMBI. *Nature.* 1999; 401:480–485. [PubMed: 10519551]
17. Conley CA, Silburn R, Singer MA, Ralston A, Rohwer-Nutter D, Olson DJ, Gelbart W, Blair SS. Crossveinless 2 contains cysteine-rich domains and is required for high levels of BMP-like activity during the formation of the cross veins in *Drosophila*. *Development.* 2000; 127:3947–3959. [PubMed: 10952893]

- 18. Serpe M, Umulis D, Ralston A, Chen J, Olson DJ, Avanesov A, Othmer H, O'Connor MB, Blair SS. The BMP-binding protein Crossveinless 2 is a short-range, concentration-dependent, biphasic modulator of BMP signaling in *Drosophila*. *Dev Cell*. 2008; 14:940–953. This study in *Drosophila* combines genetics, biochemistry, and mathematical modeling to analyze the pro- and anti-BMP activity of CV2. [PubMed: 18539121]
- 19. Zhang JL, Qiu LY, Kotsch A, Weidauer S, Patterson L, Hammerschmidt M, Sebald W, Mueller TD. Crystal structure analysis reveals how the Chordin family member crossveinless 2 blocks BMP-2 receptor binding. *Dev Cell*. 2008; 14:739–750. First report of the crystal structure of a BMP/CR domain complex. [PubMed: 18477456]
- 20. Kelley R, Ren R, Pi X, Wu Y, Moreno I, Willis M, Moser M, Ross M, Podkowa M, Attisano L, et al. A concentration-dependent endocytic trap and sink mechanism converts Bmp from an activator to an inhibitor of Bmp signaling. *J Cell Biol*. 2009; 184:597–609. This paper demonstrates that when CV2 is in molar excess to BMP ligands it induces their removal by endocytosis. It also shows that Noggin, but not Chordin, behaves similarly. [PubMed: 19221194]
- 21. Ambrosio AL, Taelman VF, Lee HX, Metzinger CA, Coffinier C, De Robertis EM. Crossveinless-2 is a BMP feedback inhibitor that binds Chordin/BMP to regulate *Xenopus* embryonic patterning. *Dev Cell*. 2008; 15:248–260. The “sink” model for the flow of Chd/BMP complexes towards CV2 in the ventral center of the *Xenopus* gastrula was first proposed in this study. Chordin binds CV2 with affinities in the low nanomolar range. This flow mechanism explains the pro-BMP effects of CV2. [PubMed: 18694564]
- 22. Zhang JL, Patterson LJ, Qiu LY, Graziussi D, Sebald W, Hammerschmidt M. Binding between Crossveinless-2 and Chordin von Willebrand factor type C domains promotes BMP signaling by blocking Chordin activity. *PLoS One*. 2010; 5:e12846. [PubMed: 20886103]
- 23. Piccolo S, Agius E, Lu B, Goodman S, Dale L, De Robertis EM. Cleavage of Chordin by Xolloid metalloprotease suggests a role for proteolytic processing in the regulation of Spemann organizer activity. *Cell*. 1997; 91:407–416. [PubMed: 9363949]
- 24. Marques G, Musacchio M, Shimell MJ, Wunnenberg-Stapleton K, Cho KW, O'Connor MB. Production of a DPP activity gradient in the early *Drosophila* embryo through the opposing actions of the SOG and TLD proteins. *Cell*. 1997; 91:417–426. [PubMed: 9363950]
- 25. Ferguson EL, Anderson KV. Localized enhancement and repression of the activity of the TGF-beta family member, decapentaplegic, is necessary for dorsal-ventral pattern formation in the *Drosophila* embryo. *Development*. 1992; 114:583–597. This classic genetic study described an extensive allelic series of BMP phenotypes in *Drosophila tolloid* mutants, suggesting that Tolloid provided the rate-limiting step in D-V patterning. [PubMed: 1618130]
- 26. Inomata H, Haraguchi T, Sasai Y. Robust stability of the embryonic axial pattern requires a secreted scaffold for chordin degradation. *Cell*. 2008; 134:854–865. This study reports the discovery of a new player in the BMP pathway. Ont-1, a member of the Olfactomedin protein family, acts as scaffold protein that bridges the binding of the Chordin substrate to its proteolytic enzyme Tolloid. [PubMed: 18775317]
- 27. Lee HX, Ambrosio AL, Reversade B, De Robertis EM. Embryonic dorsal-ventral signaling: secreted frizzled-related proteins as inhibitors of tolloid proteinases. *Cell*. 2006; 124:147–159. [PubMed: 16413488]
- 28. Muraoka O, Shimizu T, Yabe T, Nojima H, Bae YK, Hashimoto H, Hibi M. Sizzled controls dorso-ventral polarity by repressing cleavage of the Chordin protein. *Nat Cell Biol*. 2006; 8:329–338. [PubMed: 16518392]
- 29. Ploper D, Lee HX, De Robertis EM. Dorsal-ventral patterning: Crescent is a dorsally secreted Frizzled-related protein that competitively inhibits Tolloid proteases. *Dev Biol*. 2011; 352:317–328. [PubMed: 21295563]
- 30. Lee HX, Mendes FA, Plouhinec JL, De Robertis EM. Enzymatic regulation of pattern: BMP4 binds CUB domains of Tolloids and inhibits proteinase activity. *Genes Dev*. 2009; 23:2551–2562. This study provides the answer for an old mystery: why the BMP1/Tolloid metalloproteinase copurified with Bone Morphogenetic Proteins extracted from decalcified bone extracellular matrix. It was found that BMP4 binds to the CUB domains of Tolloid/BMP1, inhibiting its activity in a non-competitive manner. [PubMed: 19884260]

31. Ramirez-Weber FA, Kornberg TB. Cytonemes: cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell*. 1999; 97:599–607. [PubMed: 10367889]
32. Greco V, Hannus M, Eaton S. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell*. 2001; 106:633–645. [PubMed: 11551510]
33. Roy S, Hsiung F, Kornberg TB. Specificity of *Drosophila* cytonemes for distinct signaling pathways. *Science*. 2011; 332:354–358. [PubMed: 21493861]
34. Yan D, Lin X. Shaping morphogen gradients by proteoglycans. *Cold Spring Harb Perspect Biol*. 2009; 1:a002493. [PubMed: 20066107]
35. Giraldez AJ, Copley RR, Cohen SM. HSPG modification by the secreted enzyme Notum shapes the Wingless morphogen gradient. *Dev Cell*. 2002; 2:667–676. [PubMed: 12015973]
- 36. Wang X, Harris RE, Bayston LJ, Ashe HL. Type IV collagens regulate BMP signalling in *Drosophila*. *Nature*. 2008; 455:72–77. This paper introduces a new extracellular component of the BMP pathway, Collagen IV. It discovered how Collagen IV, Twisted gastrulation, and Chordin interact in the extracellular matrix to control BMP diffusion and signaling. [PubMed: 18701888]
37. Vuilleumier R, Springhorn A, Patterson L, Koidl S, Hammerschmidt M, Affolter M, Pyrowolakis G. Control of Dpp morphogen signalling by a secreted feedback regulator. *Nat Cell Biol*. 2010; 12:611–617. [PubMed: 20453847]
38. Jones CM, Armes N, Smith JC. Signalling by TGF-beta family members: short-range effects of Xnr-2 and BMP-4 contrast with the long-range effects of activin. *Curr Biol*. 1996; 6:1468–1475. [PubMed: 8939607]
39. Ohkawara B, Iemura S, ten Dijke P, Ueno N. Action range of BMP is defined by its N-terminal basic amino acid core. *Curr Biol*. 2002; 12:205–209. [PubMed: 11839272]
- 40. Marjoram L, Wright C. Rapid differential transport of Nodal and Lefty on sulfated proteoglycan-rich extracellular matrix regulates left-right asymmetry in *Xenopus*. *Development*. 2011; 138:475–485. This paper provides evidence that sulfated proteoglycans facilitate the movement over long distances of the left-right Nodal morphogen, a TGFβ ligand, in *Xenopus* embryos. [PubMed: 21205792]
41. Little SC, Mullins MC. Extracellular modulation of BMP activity in patterning the dorsoventral axis. *Birth Defects Res C Embryo Today*. 2006; 78:224–242. [PubMed: 17061292]
42. Olivares GH, Carrasco H, Aroca F, Carvallo L, Segovia F, Larrain J. Syndecan-1 regulates BMP signaling and dorso-ventral patterning of the ectoderm during early *Xenopus* development. *Dev Biol*. 2009; 329:338–349. [PubMed: 19303002]
43. Dutko JA, Mullins MC. SnapShot: BMP Signaling in Development. *Cell*. 2011; 145:636–636.e632. [PubMed: 21565618]
44. Kuo WJ, Digman MA, Lander AD. Heparan sulfate acts as a bone morphogenetic protein coreceptor by facilitating ligand-induced receptor hetero-oligomerization. *Mol Biol Cell*. 2010; 21:4028–4041. [PubMed: 20861306]
45. Dale L, Wardle FC. A gradient of BMP activity specifies dorsal-ventral fates in early *Xenopus* embryos. *Semin Cell Dev Biol*. 1999; 10:319–326. [PubMed: 10441546]
46. De Robertis EM. Spemann's organizer and self-regulation in amphibian embryos. *Nat Rev Mol Cell Biol*. 2006; 7:296–302. [PubMed: 16482093]
47. Reversade B, De Robertis EM. Regulation of ADMP and BMP2/4/7 at opposite embryonic poles generates a self-regulating morphogenetic field. *Cell*. 2005; 123:1147–1160. [PubMed: 16360041]
48. De Robertis EM. Spemann's organizer and the self-regulation of embryonic fields. *Mech Dev*. 2009; 126:925–941. [PubMed: 19733655]
49. Eldar A, Dorfman R, Weiss D, Ashe H, Shilo BZ, Barkai N. Robustness of the BMP morphogen gradient in *Drosophila* embryonic patterning. *Nature*. 2002; 419:304–308. [PubMed: 12239569]
- 50. Wang YC, Ferguson EL. Spatial bistability of Dpp-receptor interactions during *Drosophila* dorsal-ventral patterning. *Nature*. 2005; 434:229–234. This study, and the following one, showed that BMPs are transported over long distances in the perivitelline space of the *Drosophila* blastoderm to generate peak levels of BMP signaling. [PubMed: 15759004]

51. Shimmi O, Umulis D, Othmer H, O'Connor MB. Facilitated transport of a Dpp/Scw heterodimer by Sog/Tsg leads to robust patterning of the Drosophila blastoderm embryo. *Cell*. 2005; 120:873–886. [PubMed: 15797386]
- 52. Zakin L, Chang EY, Plouhinec JL, De Robertis EM. Crossveinless-2 is required for the relocalization of Chordin protein within the vertebral field in mouse embryos. *Dev Biol*. 2010; 347:204–215. Analyzing protein localization in the mouse embryo, this study showed that in the vertebral field Chordin protein secreted by the intervertebral disc is transported to the vertebral body. This relocalization is entirely dependent on the presence of CV2. [PubMed: 20807528]
- 53. Turing AM. The Chemical Basis of Morphogenesis. *Philos Trans Royal Soc London*. 1952; 237:37–72. In this must-read classic, mathematician Alan Turing proposed the term morphogen for the signals that control development. His diffusion-reaction equations explain how pattern is formed, for example in gastrulation, according to the well-known physical laws of diffusion.
54. Gierer A, Meinhardt H. A theory of biological pattern formation. *Kybernetik*. 1972; 12:30–39. [PubMed: 4663624]
55. Meinhardt H. Models for the generation and interpretation of gradients. *Cold Spring Harb Perspect Biol*. 2009; 1:a001362. [PubMed: 20066097]
- 56. Ben-Zvi D, Shilo BZ, Fainsod A, Barkai N. Scaling of the BMP activation gradient in Xenopus embryos. *Nature*. 2008; 453:1205–1211. This study used reaction-diffusion equations and experimental approaches to show that the flow of Chordin/BMP complexes is essential for the scaling or size-adjustment of the gastrula morphogenetic field. [PubMed: 18580943]
57. Zhang JL, Huang Y, Qiu LY, Nickel J, Sebald W. von Willebrand factor type C domain-containing proteins regulate bone morphogenetic protein signaling through different recognition mechanisms. *J Biol Chem*. 2007; 282:20002–20014. [PubMed: 17483092]
- 58. Paulsen M, Legewie S, Eils R, Karaulanov E, Niehrs C. Negative feedback in the bone morphogenetic protein 4 (BMP4) synexpression group governs its dynamic signaling range and canalizes development. *Proc Natl Acad Sci U S A*. 2011; 108:10202–10207. This experimental and modeling study explains how feedback inhibitors – BAMBI, Smad6 and Smad7 – expressed in the BMP4 synexpression group linearize the response and reduce the variability to BMP signals. [PubMed: 21633009]
59. Lander AD. Pattern, growth, and control. *Cell*. 2011; 144:955–969. [PubMed: 21414486]
60. Ben-Zvi D, Barkai N. Scaling of morphogen gradients by an expansion-repression integral feedback control. *Proc Natl Acad Sci U S A*. 2010; 107:6924–6929. [PubMed: 20356830]
61. Spemann H, Mangold H. Induction of embryonic primordial by implantation of organizers from a different species. *Roux's Arch Entw Mech*. 1924; 100:599–638. (reprinted and translated in *Int J Dev Biol* 2001, 45:13–31).
62. Umulis DM, Shimmi O, O'Connor MB, Othmer HG. Organism-scale modeling of early Drosophila patterning via bone morphogenetic proteins. *Dev Cell*. 2010; 18:260–274. [PubMed: 20159596]
63. Wartlick O, Mumcu P, Kicheva A, Bittig T, Seum C, Julicher F, Gonzalez-Gaitan M. Dynamics of Dpp signaling and proliferation control. *Science*. 2011; 331:1154–1159. [PubMed: 21385708]
64. Eivers E, Fuentealba LC, De Robertis EM. Integrating positional information at the level of Smad1/5/8. *Curr Opin Genet Dev*. 2008; 18:304–310. [PubMed: 18590818]
65. Massague J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev*. 2005; 19:2783–2810. [PubMed: 16322555]

Figure 1.

Schematic representation of the biochemical pathway controlling *Xenopus* dorsal-ventral differentiation. Components of the extracellular space are depicted according to their dorsal or ventral localization within the embryo. (1) Chordin and Noggin are BMP antagonists secreted by the dorsal center [10–12]. (2) Chordin forms together with Tsg and BMP a trimolecular complex [13]. (3) Ont1 enhances the binding between the substrate Chordin and its proteolytic enzyme Tolloid [26**]. Tolloid activity is competitively inhibited by Sizzled, Crescent, and non-competitively by BMPs (see boxes) [27–29,30**]. (4) The Chd/BMP/Tsg complex flows towards the ventral side, (5) where it binds to CV2 which acts as a Chd/BMP sink [21**]. Upon digestion of Chordin by Tolloid [23], (6) CV2-bound BMPs are transferred to the receptor complex which phosphorylates Smad1/5/8 transcription factors. Smad1/5/8 transduce the BMP signal, bind to a co-Smad (Smad4), and translocate to the nucleus to activate transcription [65]. (7) Proteolytic fragments of Chordin also bind CV2 [21**], which presumably clears them by endocytosis [20*]. (8) The pseudo-receptor BAMBI binds BMPs and inhibits formation of the BMP receptor complex [16].

Figure 2.

Extracellular network of proteins involved in the self-regulation of *Xenopus* dorsal-ventral patterning. Black arrows indicate protein-protein interactions, blue arrows transcriptional regulations, and red arrows the direction of the flow of Chordin-BMP complexes from the dorsal to the ventral side of the embryo. The resulting BMP activity gradient is indicated on the right. The rate-limiting reaction in this network is that of Tolloid, a zinc metalloproteinase that cleaves Chordin, releasing BMPs from its inactive complexes [23]. Tolloid activity is regulated by the competitive inhibitors emanating from the dorsal (Crescent [29]) and ventral (Sizzled [27]) centers, as well as by BMP4 which acts as a non-competitive inhibitor of Tolloid on the ventral side [30**]. The behavior of the entire biochemical pathway can be modeled using reaction-diffusion equations. Mathematical analyses have shown that the flow of BMPs from dorsal towards ventral regions provides robustness to the system [56**], and that the Chordin substrate, which is produced in very large amounts, is a major inhibitor of Tolloid activity on the dorsal side [30**].

Figure 3.

Chordin relocalization in the vertebral field of the mouse embryo requires CV2. (a) At 12 days post coitum, *Chd* mRNA is transcribed (blue staining) in the intervertebral disc (ivd) but its protein (brown staining) accumulates in the vertebral body (vb). This indicates a relocalization of Chd protein, whereas (b) CV2 transcripts and protein co-localize in the vertebral body (histological sections at the lumbar L4 and L5 level). (c, d) Chd accumulation in the vertebral body of wild-type embryos is lost in CV2^{-/-} mutant embryos, in which Chordin is found instead in the intervertebral disc (arrow). This shows that CV2 is required for the relocalization of Chd protein from the intervertebral disc, where it is transcribed, to the vertebral body (sections at the thoracic T3 and T4 level). (e) Schematic model of the developing vertebral field. CV2 is retained in the vertebral body through his interaction with Heparan Sulfate Proteoglycans (such as Glypican) and acts as a sink for Chd/BMP complexes produced in the intervertebral disc. Upon cleavage of Chd by Tolloid metalloproteinases, BMPs are released in the vertebral body producing the high-BMP signals required for cartilage differentiation. Adapted from [52*], with permission.

Figure 4.

The frog dorsal center can induce epidermal differentiation at a distance. (a) *Cytokeratin* mRNA marks the epidermis, a region of high BMP signaling in the *Xenopus* embryo at neural plate stage. (b) When four BMPs are depleted by knocking down simultaneously BMP2/4/7 and ADMP with antisense Morpholino oligonucleotides, the entire ectoderm is converted to central nervous system tissue as indicated by the absence of *Cytokeratin* mRNA staining. (c) Transplantation of a wild-type (WT) dorsal center into an embryo depleted of BMPs restores dorsal-ventral patterning and epidermal differentiation at a distance. The transplanted wild-type tissue, which was labeled with the lineage tracer nuclear-LacZ, gives rise to notochord. These experiments show that the dorsal center is a source of BMP ligands that are transported and induce BMP signaling at a distance. Adapted from [47], with permission.