REVIEW SUMMARY

EMBRYO DEVELOPMENT

# BMP gradients: A paradigm for morphogen-mediated developmental patterning

Ethan Bier* and Edward M. De Robertis*

**BACKGROUND:** Classic embryological studies showed that diffusible factors (morphogens) influence cell fate during dorsal-ventral (DV) axis patterning. Subsequently, mathematical analyses applied reaction-diffusion equations in a theoretical framework to model how stable gradients of morphogenetic factors might be created in developing cell fields, according to the laws of physical chemistry. This work suggested mechanisms by which such gradients form and are read in a threshold-dependent fashion to establish distinct cellular responses. As highlighted in this Review, these pioneering experimental and intellectual insights laid the groundwork for more recent studies that have elucidated the mechanisms by which morphogen gradients are generated and stabilized by molecular feedback circuits.

**ADVANCES:** The molecular players involved in early DV patterning uncovered over the past two decades constitute a highly conserved cohort of extracellular factors that regulate bone morphogenetic protein (BMP) signaling. A key insight was the identification of the homologous proteins Short gastrulation (Sog) and Chordin as BMP-binding proteins in *Drosophila* and *Xenopus* 20 years ago. Since then, analysis of this patterning system has led to dramatic advances in our understanding of the molecular mechanisms regulating early DV axis specification. Elements of this pathway include secreted BMP ligands and BMP antagonists, as well as extracellular metalloproteinases that cleave and inactivate BMP antagonists. Identification of these and other accessory proteins provided strong support for the proposal that an inversion of the DV axis had occurred between arthropods and vertebrates. Analysis of how these components are deployed in an array of species with divergent developmental strategies has deepened our understanding of this ancestral DV patterning biochemical pathway. These comparative studies have shed light on the broader question of a how a conserved core pathway can be modified during evolution to accommodate different forms of embryogenesis while maintaining common output effector functions. In addition, advances in computational analysis have provided the necessary tools to analyze BMP-mediated signaling in quantitative terms and have provided important insights into how this patterning process is integrated with cell proliferation and tissue growth. One such insight is the identification of expanders (such as Pentagone and Sizzled), which are secreted molecules typically produced at the low end of a gradient that stabilize the ligand, scaling the gradient to the growth of tissues.

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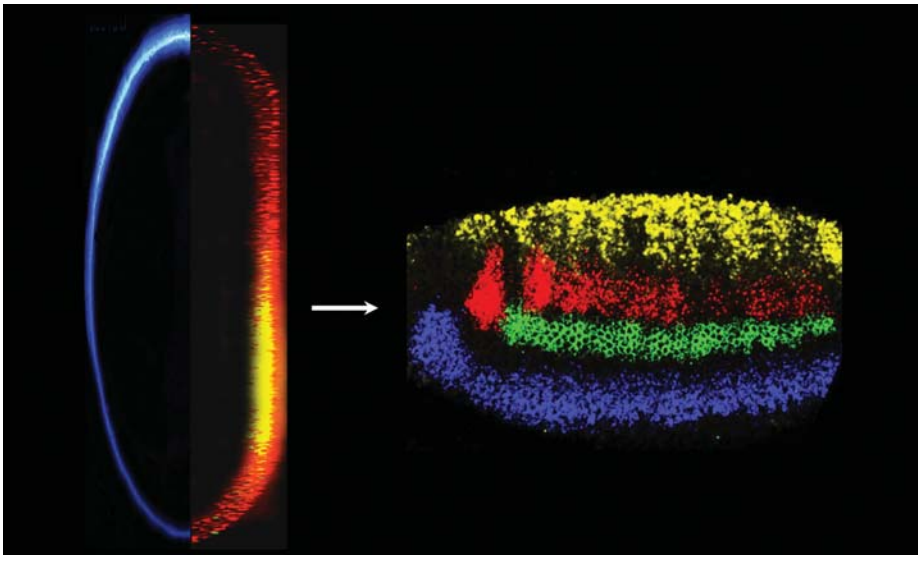
**OUTLOOK:** An important unanswered question is how morphogen gradients form and function reliably in the face of intrinsic signal-degrading processes to achieve consistent developmental patterning and growth. One testable hypothesis, based on the "wisdom of crowds" concept, that may shed light on this challenging problem is that several independent features of morphogen gradients can be read in parallel by cells and can also serve as inputs to an array of feedback modules that integrate instantaneous levels of signaling, perform time averaging of signals, and act locally to coordinate signaling between neighboring cells. A consensus-based estimate of the relative position of a cell may be reached by deploying multiple parallel feedback modules. In addition, it will be important to determine the roles of mechanisms, such as free or facilitated diffusion in the extracellular space; exosomes; and cytonemes in morphogen gradient function. Understanding the mechanisms by which morphogen-mediated patterning systems evolve to maintain key elements of overall body design while allowing for a marked diversity in the spatial deployment of various subsets of signaling components is another compelling challenge. Such studies should better illuminate the precise nature of highly constrained developmental processes and delineate more fluid features of the networks that permit remodeling of core components to meet the specialized selective needs of particular organisms. These future studies should refine and strengthen one of the best paradigms for understanding development. ■



**Conserved BMP-mediated patterning of the DV axis.** Gradients of proteins in vertebrates (left: blue Chordin stain) and invertebrates (left: red/yellow Sog stain) initiate patterning along the DV axis. These gradients are then read to establish distinct zones of gene expression within the central nervous system (right: *dpp*, yellow; *msh*, red; *ind*, green; *vnd*, blue).

CREDITS: CHORDIN GRADIENT (LEFT) COLORED AND MODIFIED BY PHOTOSHOP FROM FIG. 3A OF J. L. PLOUHINEC *ET AL.*, *PROC. NATL. ACAD. SCI. U.S.A.* **110**, 20372–20379 (2013); SOG GRADIENT (RIGHT) MODIFIED BY PHOTOSHOP FROM FIG. 1B″ OF S. SRINIVASAN *ET AL.*, *DEV. CELL* **2**, 91–101 (2002)

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REVIEW

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# BMP gradients: A paradigm for morphogen-mediated developmental patterning

Ethan Bier[1]* and Edward M. De Robertis[2,3]*

**Bone morphogenetic proteins (BMPs) act in dose-dependent fashion to regulate cell fate choices in a myriad of developmental contexts. In early vertebrate and invertebrate embryos, BMPs and their antagonists establish epidermal versus central nervous system domains. In this highly conserved system, BMP antagonists mediate the neural-inductive activities proposed by Hans Spemann and Hilde Mangold nearly a century ago. BMPs distributed in gradients subsequently function as morphogens to subdivide the three germ layers into distinct territories and act to organize body axes, regulate growth, maintain stem cell niches, or signal inductively across germ layers. In this Review, we summarize the variety of mechanisms that contribute to generating reliable developmental responses to BMP gradients and other morphogen systems.**

A major question in developmental biology is how information provided in a fertilized egg can trigger the chain of events leading cells in the embryo to adopt different developmental fates and to do so with great reliability. Classic fate-mapping studies revealed that cells in different regions of the embryo predictably give rise to specific tissues or organs. But how is this diversification of cell potential achieved in a self-regulating system? Hans Spemann addressed this question by conducting a series of illuminating experiments in which he transplanted tissue fragments between donor and recipient amphibian embryos of different pigmentation. Most transplants resulted in the cells adopting the fate of the surrounding cells of the recipient (for example, neural plate or epidermis). However, embryos that received a graft of the dorsal blastopore lip, which Spemann later named the organizer, developed a twinned (or secondary) body axis, indicating that there was something special about this region (Fig. 1, A and B). An important question raised by these early embryological experiments (*1*), which was subsequently addressed by Spemann's graduate student Hilde Mangold, was how did the transplanted organizer tissue lead to such whole-scale reprogramming of the embryo? Did the transplanted cells change fate to give rise to different cells comprising the full duplicated axis or did they alter the behavior of neighboring recipient cells? By using pigmented and unpigmented amphibian eggs as donors and hosts, Mangold demonstrated that unpigmented donor dorsal tissue gave rise to notochord in the duplicated axis, as it would ordinarily do in an undisturbed embryo (*2*) (Fig. 1B). However, ectodermal derivatives, including the central nervous system (CNS), were derived from host tissue, which suggested that the transplanted mesoderm cells produced signals that redirected the developmental trajectories of adjacent host cells. Subsequent "tissue sandwich" experiments (in which ectodermal cells were brought into contact with various mesodermal derivatives) revealed that these hypothetical diffusible signals capable of inducing a secondary neural axis were elaborated by dorsal mesodermal cells. These signals were termed "neural-inducing" factors. Spemann received the Nobel Prize for medicine in 1935. Tragically, Hilde Mangold died in a kitchen stove accident in 1924 and so was not recognized for her research.

In the early 1990s, vertebrate neural-inducing signals produced by Spemann's organizer were identified and found to act by inhibiting the bone morphogenetic protein (BMP) signaling pathway. Parallel studies in the fruitfly similarly identified conserved BMP signaling elements essential for patterning the dorsal-ventral (DV) axis [reviewed in (*3*, *4*)]. Together, these breakthroughs established a paradigm for studying diffusible developmental signals.

## Morphogens

The British mathematician Alan Turing, who cracked the Nazi Enigma code during World War II and later developed the theoretical framework for computers, proposed that information to generate complex anatomical structures might be provided by the diffusion of hypothetical substances he called "morphogens" (*5*). Turing formulated a general partial differential equation to quantitatively describe the changes in the concentration of a morphogen over time (Fig. 1C): Following Fick's law of diffusion, this equation states that the change in morphogen concentration ($C$) over time ($\delta t$) is proportional to its diffusion rate ($D$) and to the second derivative in space of the morphogen concentration ($\nabla^2 C$). In addition, the change in morphogen concentration is a function ($F$) of all of the chemical reactions it undergoes (e.g., synthesis, degradation, and association or dissociation with other proteins such as antagonists).

From this initial insight, many reaction-diffusion computer models have been derived to explain the behavior of developing systems. Francis Crick considered a special case in which one group of cells (the source) secretes a factor that diffuses into adjacent regions (the sink), where it is either counteracted or degraded (*6*) (Fig. 1E). Crick proposed that this so-called adjacent source-sink configuration can create concentration gradients of the morphogen, whose shapes remain stable over time. These gradients could then exert various effects on developing cells. Another key advance was the realization that a pair of morphogens composed of an activator and an inhibitor can generate stable patterns (*7*), provided that they originate from the same source and that the inhibitor is more diffusible (Fig. 1D). The activator turns on its own production and also the synthesis of the inhibitor, which in turn represses the activator. Stable patterns result because the inhibitor diffuses faster than the activator, turning it off in the periphery. It is amazing that these powerful mathematical frameworks for understanding long-range reaction-diffusion of morphogens were offered at a time when the chemical nature of not even a single morphogen was known. Many of these principles have now been confirmed by the BMP/Dpp and Chordin/Sog morphogenetic system (see below).

## French flag model

Lewis Wolpert suggested a simple visually evocative idea, often referred to as the "French flag model," for how a morphogen gradient can subdivide a field of otherwise equivalent cells into distinct regions (*8*) (Fig. 1F). In this model, morphogens act in a threshold-dependent fashion to control expression of distinct sets of genes in broad zones, each domain corresponding to a fixed range of morphogen level. These primary response genes in turn specify particular cell fates (e.g., transcription factors) or trigger secondary patterning events by signaling to adjacent domains (e.g., secreted signals). Important mechanistic questions for such models are how cells detect abrupt threshold levels of the morphogen and then how they execute distinct responses in a coherent fashion. One general feature of many primary response genes that helps resolve borders is cross-inhibition between factors produced in neighboring domains. Although such reciprocal inhibitory interactions can act as a toggle switch to convert smooth gradients into

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sharp on-off responses, it remains to be determined how given thresholds are accurately read from individual to individual and how "salt-and-pepper" responses are avoided along borders within a given embryo. As discussed in more detail below, several additional homeostatic mechanisms are likely to be at play, including: differential timing of gene responses; transiently acting prepatterns that bias cells to respond differently to a given level of morphogen; growth of the tissue (with or without corresponding changes in the length scale of the gradient); the ability of cells to read other aspects of a gradient, including its slope; inflections in the gradient (second derivative terms); integrated effects of the gradient; and noise, which can trigger differential signaling between neighboring cells at the tail end of a gradient.

## BMP-mediated patterning of the embryonic DV axis

Two of the most noteworthy and well-studied examples of conservation of developmental patterning mechanisms are specification of segmental identities along the anterior-posterior (AP) axis by *Hox* genes (*9*) and subdivision of the DV axis into distinct ectodermal domains by graded BMP signaling (*10*). The notable homologies in DV patterning were first revealed through comparisons of this process in *Drosophila* and *Xenopus*, and informative variations on this theme have subsequently been provided by analysis of a broad range of animal species (see below).

### *All-or-none BMP signaling during Drosophila neural induction*

In *Drosophila*, DV patterning is initiated by a ventral-to-dorsal gradient of the maternally provided morphogen Dorsal (Dl), an NFκB-related transcription factor that specifies mesoderm (e.g., somatic muscle, heart) at high levels, neuroectoderm (e.g., ventral epidermis and CNS) at moderate levels, and dorsal epidermis and an extra-embryonic tissue known as the amnioserosa by its absence (*11*). Primary Dl response

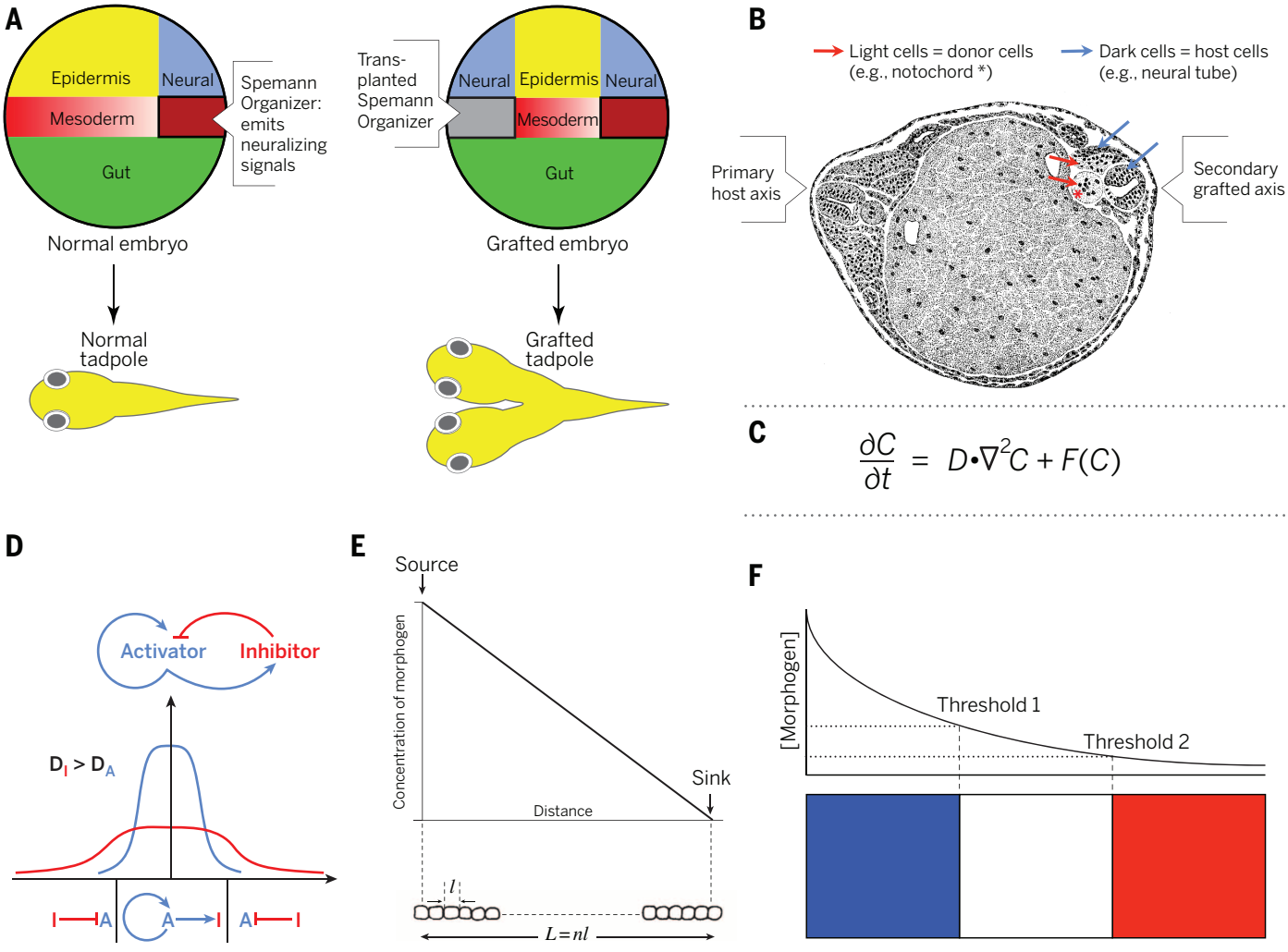
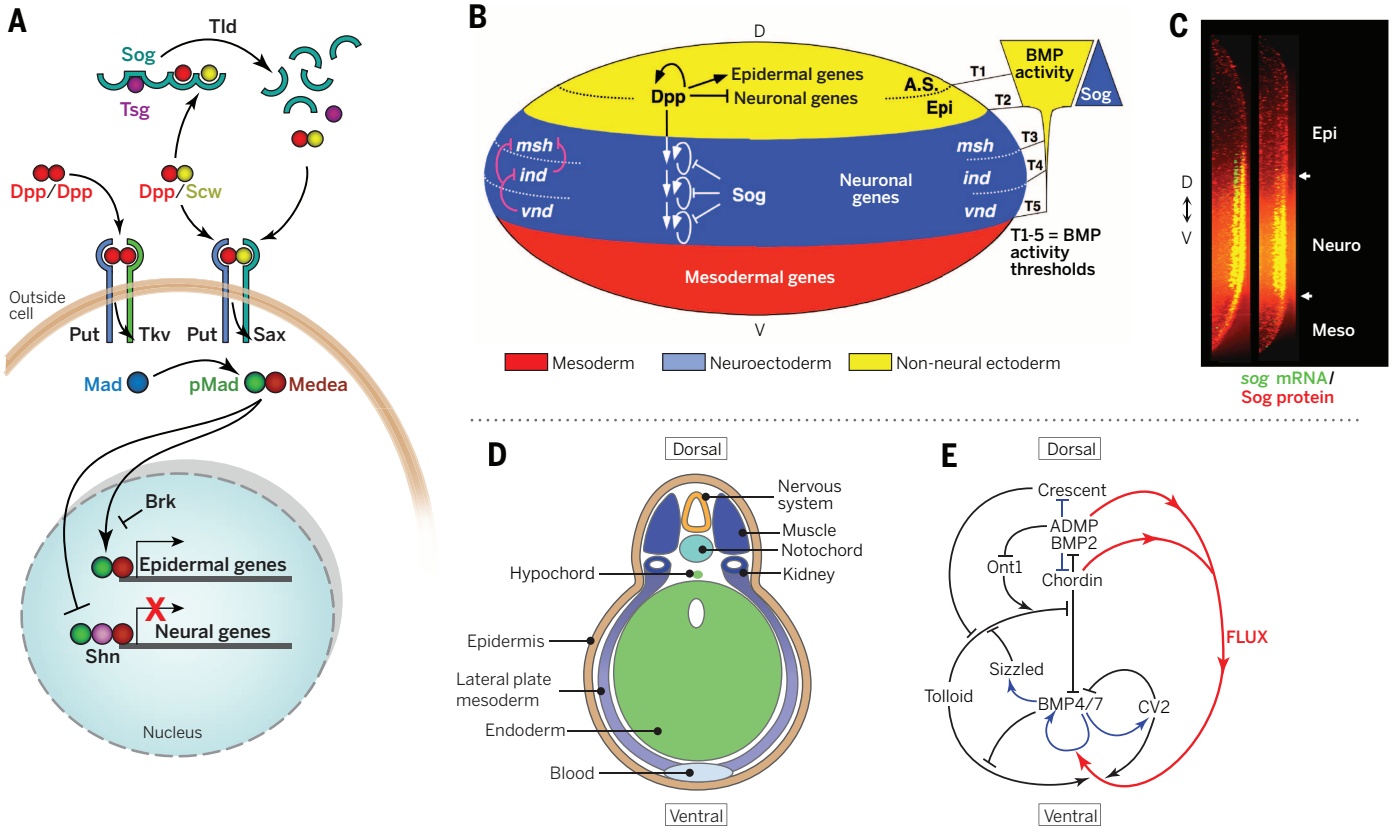
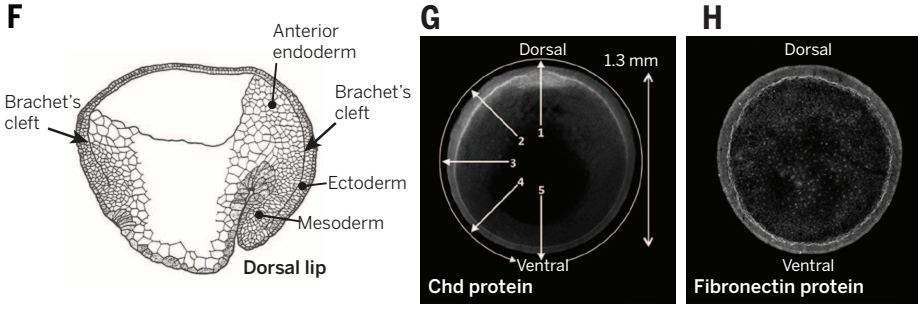



**Fig. 1. Morphogens.** (**A**) Diagram of organizer graft experiment. The gray area indicates the ventral mesodermal location of the Spemann organizer graft that leads to embryos developing duplicated neural axes. Grafts of tissue taken from other regions of the embryo typically had little, if any, effect. (**B**) Transplantation of the dorsal mesoderm (Spemann organizer) dissected from the blastopore of a donor embryo into the ventral mesoderm of a host embryo results in an embryo with a double axis. In the induced secondary axis, the lightly pigmented donor cells (red arrows) generate mesodermal structures (notochord: asterisk and somites), whereas host tissue forms the nervous system (blue arrows). (**C**) Turing's basic reaction-diffusion equation describing how a morphogen is governed by Fick's law (see text). *C*, morphogen concentration; *t*, time; *D*, diffusion rate; $\nabla^2 C$, second derivative in space of the morphogen concentration; *F*, function. (**D**) Formation of a stable morphogen gradient via a reaction-diffusion mechanism based on two factors [activator (A) and inhibitor (I)]. The activator increases the level of the inhibitor, whereas the inhibitor negatively turns off the activator and is more diffusible than the activator. $D_I$, diffusion coefficient of inhibitor; $D_A$, diffusion coefficient of activator. (**E**) A source and sink of a morphogen can create a stable concentration gradient (as proposed by F. Crick). *L*, full length of tissue; *n*, number of cells; *l*, size of a single cell. (**F**) French flag model in which two different threshold concentrations of a morphogen elicit three distinct responses. [Credits: (A) redrawn from figure 5.3 of (*114*); (B) figure 24 of (*2*); (D) figure 1 of (*40*); (E) figure 1 of (*6*)]

genes in dorsal and lateral regions of the embryo include genes involved in establishing a gradient of BMP signaling.

As summarized in Fig. 2A, two *Drosophila* BMP-related proteins—Decapentaplegic (Dpp, a BMP2/4 ortholog) and Screw (Scw, a BMP5/7 homolog)—signal via heterotetrameric BMP receptors to phosphorylate and activate the transcription factor Mad in the cytoplasm (or SMADs



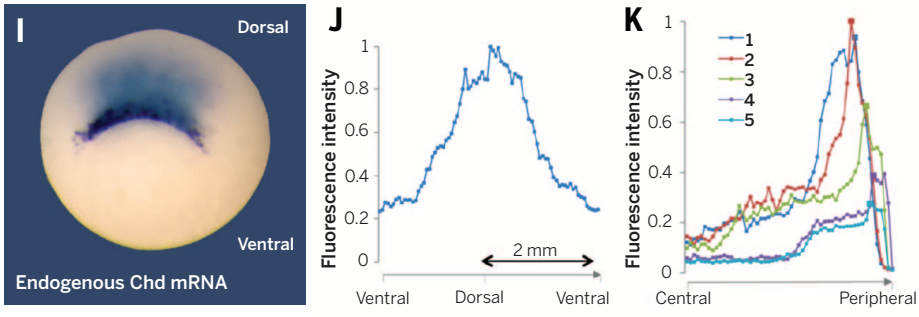




**Fig. 2. BMP patterning during neural induction in *Drosophila* and *Xenopus*.** (**A**) Diagram of BMP signaling components in *Drosophila*. See text for details. (**B**) Diagram of an early blastoderm-stage *Drosophila* embryo showing subdivision of the DV axis into mesoderm, neuroectoderm, and epidermis. (Right) A high-level BMP activity gradient in dorsal Dpp-expressing cells is created in response to an inverse Sog gradient (emanating from the neuroectoderm) that subdivides the epidermal region into amnioserosa and epidermis proper (thresholds T1 and T2, respectively). A low-level BMP gradient in the neuroectoderm helps partition this region into the three domains expressing the neural identity genes *msh*, *ind*, and *vnd* (thresholds T3 to T5, respectively). (Left) The neural identity genes engage in ventral-dominant repression wherein Vnd inhibits *ind* and *msh* and Ind inhibits *msh*. (**C**) Sog protein gradient viewed in cross section at two successive stages in the blastoderm embryo (left, when Sog protein is first expressed; right, 15 min later). (**D**) Stereotypical DV tissue types of the vertebrate body plan. (**E**) BMP activity along the DV axis results from a series of direct protein-protein interactions between Chordin and other partners (black arrows), transcriptional regulation (blue arrows), and protein flux (red arrows). The entire embryo participates in forming the BMP gradient, which results from the dueling activities of the dorsal and ventral signaling centers. (**F**) Diagram of a sagittal section of a *Xenopus* gastrula (as proposed by P. Nieuwkoop). Brachet's cleft is the narrow cavity that separates the mesendoderm from the ectoderm, encircling the entire DV axis (arrows). (**G**) The Chordin protein gradient spans the entire DV axis. (**H**) Distribution of Fibronectin protein in a comparable embryo is uniform. (**I**) Chordin mRNA is transcribed only on the dorsal side. (**J**) Protein quantification along the DV Brachet's cleft; the gradient forms over 2 mm. (**K**) Chordin fluorescence in radial tracings along the numbered arrows indicated in (G). [Credits: (C) figure 1, b′ and b″, of (*22*); (E to K) from (*40*)]

in vertebrates). Phosphorylated Mad (pMAD ≈ vertebrate pSMAD1/5/8) complexes with a related cofactor Medea (≈ vertebrate SMAD4), enters the nucleus, and activates some target genes (e.g., epidermal genes activated by binding of Mad/Medea complexes to cis-regulatory DNA sequences) but represses expression of other genes [e.g., neuronal genes repressed via a trimeric transcriptional complex consisting of Mad, Medea, and a zinc finger protein Schnurri (Shn)] (*12*). An important feature of this dual action of BMP signaling is that activation of epidermal genes (mediated by activation elements) (*13*) requires much higher levels of BMP signaling (achieved only dorsally where BMPs are produced) than repression of neuronal genes [mediated by silencing elements (SEs)] (*11*, *12*).

Several extracellular proteins regulate BMP signaling (Fig. 2B). In the lateral neuroectoderm, the BMP antagonist short gastrulation (Sog = Chordin) is secreted. In the dorsal epidermis, Dpp, the metalloproteinase Tolloid (Tld), and a cofactor Twisted gastrulation (Tsg) are produced. BMP receptor subunits, Mad, Medea, Shn, and Scw are ubiquitously expressed, although some of these factors are up-regulated in specific patterns [reviewed in (*14*–*17*)]. Dpp and Sog play key roles within their respective domains of expression to stabilize epidermal versus neuroectodermal cell fates, respectively, and also to diffuse into adjacent domains to form gradients that influence patterning therein.

The first stage of BMP patterning in the *Drosophila* ectoderm, analogous to vertebrate neural induction, relies on the all-or-none effect of high-level BMP signaling in the dorsal epidermis to repress expression of neuronal genes in that region. Thus, in *dpp*-mutant embryos, neuroblast-specifying genes such as those of the Achaete-Scute complex are ectopically expressed in the dorsal epidermis (*18*). Strong Dpp signaling also activates expression of epidermal targets, including the *dpp* gene itself (referred to as autoactivation). Because BMP receptors are present throughout the embryo, coupled Dpp diffusion and autoactivation creates a positive-feedback loop with the potential for spreading Dpp expression invasively from the epidermis into the neuroectoderm (*19*). The BMP antagonist Sog plays a key role in protecting the neuroectoderm from such Dpp invasion by preventing BMP signaling from reaching the high levels required to trigger autoactivation (however, graded low-level BMP signaling is likely to be present in the neuroectoderm; see below). BMP signals are also blocked in the *Drosophila* neuroectoderm by the transcriptional repressor Brinker (*20*). In summary, strong BMP signaling in the *Drosophila* epidermis represses expression of neural genes, and this effect is blocked by BMP antagonists in the neuroectoderm.

### Graded high-level BMP-mediated activation patterns the dorsal epidermis

In addition to its all-or-none repression of neural genes dorsally, graded high-level BMP signaling activates nested patterns of epidermal gene expression and partitions the dorsal region into the epidermis proper (dorsolateral portion) and the amnioserosa (dorsal portion). This BMP activity gradient forms primarily in response to an inverse protein gradient of the BMP antagonist Sog. Sog is secreted from the neuroectoderm, diffuses into the dorsal region (Fig. 2, B and C), and binds preferentially to Dpp:Scw heterodimers (the most potent BMP ligand), thereby blocking BMP's access to its receptors (*21*). Creation of the Sog protein gradient (Fig. 2C), which is highest near the source of Sog and diminishes toward the dorsal midline, requires activity of the Tld protease (*22*). Tld can cleave and inactivate Sog (*23*) (and can also generate alternative forms of Sog; see below). Because Tld is expressed in dorsal cells, a classical source-sink configuration is established wherein Sog diffusing from a ventral source is degraded dorsally by Tld. Tld cleavage of Sog requires binding of Dpp:Scw, Tsg, and Sog to form a trimeric complex. This trimeric complex may help concentrate Dpp:Scw heterodimers along the dorsal midline by a shuttling mechanism wherein Sog binding to Dpp:Scw prevents receptor-mediated clearance of the ligand while at the same time transporting Dpp:Scw dorsally. Cleavage of Sog by Tld then releases Dpp:Scw to signal (*21*, *24*). In addition to these purely diffusion-based mechanisms, which are thought to occur in the thin layer of perivitelline fluid between the embryo and surrounding vitelline membrane, there is evidence for endocytosis playing a role as a sink for Sog (*22*), for extracellular matrix (ECM) interactions (*25*–*27*), intracellular regulation of SMADs via linker phosphorylation (*28*), secondary signal-dependent augmentation of BMP signaling in dorsal-most cells (*29*, *30*), and feed-forward cooperation between SMADs and the primary BMP target gene *zen* to regulate gene expression in the amnioserosa (*31*), acting in concert to steepen the BMP activity gradient.

### Graded low-level BMP-mediated repression patterns the neuroectoderm

A steep low-level BMP gradient is likely to form within the lateral neuroectoderm of the *Drosophila* embryo as a consequence of Dpp diffusing in from the dorsal epidermis and being bound and sequestered by high levels of Sog (i.e., the epidermis is the source of Dpp, and Sog is the Dpp sink in the neuroectoderm). Several types of Sog:Dpp complexes may contribute to a BMP sink, including secreted full-length Sog (preferentially binding Dpp:Scw heterodimers); truncated forms of Sog, known as "Supersog," that bind and inhibit the activity of Dpp:Dpp homodimers (*25*, *32*, *33*); and forms of Sog (or Supersog) that associate with membranes via palmitoylation of a type II secretion signal (*34*). Although this hypothetical BMP activity gradient has not been directly visualized (probably due to its very low levels), genetic evidence suggests that such a gradient plays a role in subdividing the neuroectoderm into nonoverlapping domains that give rise to three primary rows of CNS neuroblasts (*35*). Neuronal fates in these three domains are specified by so-called neural identity genes, which encode the homeobox proteins Vnd (ventral row-one neuroblasts), Ind (intermediate or medial row-two neuroblasts), and Msh (dorsal row-three neuroblasts) (Fig. 3A). Neural identity genes engage in a vectorial form of cross-inhibition wherein more ventral genes repress expression of more dorsal genes (e.g., Vnd inhibits *ind* and *msh*; Ind inhibits *msh*) (*36*) (Fig. 2B). This ventral-dominant chain of repression results in sharp mutually exclusive patterns of neural identity gene expression.

Genetic analysis of BMP patterning in the neuroectoderm under conditions where it was possible to parse the effects of this morphogen from those of Dorsal revealed that BMPs repress neural identity genes in a dose-dependent fashion such that *ind* is repressed more efficiently than *msh* (*35*). Strong repression of *ind* in dorsal neuroectodermal cells near the epidermal source of Dpp relieves Ind-dependent repression of *msh* in a dorsal-most stripe of neuroectodermal cells. Consistent with these genetic findings, biochemical studies have identified SEs mediating BMP repression in cis-regulatory modules (CRMs) of the *msh* versus *ind* genes. Mad/Med/Shn complexes bind with higher affinity to an SE site in the *ind* CRM than to those in the *msh* CRM (*37*). This difference in binding affinities is relevant in vivo because replacing an *msh* Mad/Med/Shn binding site with the *ind* site results in Dpp-dependent repression of *msh*-reporter gene expression in its normal neuroectodermal domain (*37*), providing a rare example of this direct mechanism in setting a threshold response to a morphogen.

These studies show that in *Drosophila*, the maternal Dl gradient is interpreted by the Dpp/Sog morphogens to elicit the differentiation of at least five ectodermal cell types: two in the epidermal domain that are distinguished by differing levels of high BMP signaling (amnioserosa and epidermis proper) and three subdivisions within the CNS (i.e., the Vnd, Ind, and Msh domains) giving rise to neuroblasts in rows one through three.

### DV patterning in Xenopus

A gradient of BMP and Chordin signaling also controls DV histotypes in vertebrates, coordinately determining cell differentiation in the ectoderm, mesoderm, and endoderm germ layers. Low BMP levels cause differentiation of ectoderm to CNS, intermediate levels to neural crest, and high levels to epidermis. In the mesoderm, low BMP gives rise to notochord, at slightly higher levels to skeletal muscle (in segmental structures called somites), then kidney (each segment develops a kidney tubule in the embryo); lateral plate (which gives rise to the body wall); and, at the highest BMP levels, to blood (Fig. 2D). DV differentiation of the endoderm is similarly regulated. These tissues represent the invariant body plan shared by all vertebrates. This raises the question of how many morphogen gradients exist. Is there one gradient per germ layer? How would each gradient be regulated coordinately so that a perfectly harmonious embryo is formed every time? The mechanisms involved are self-organizing because if blastula embryos are cut in half, the part containing the organizer can

rescale into a well-proportioned embryo, or, if cut sagittally, the entire missing half can regenerate, forming identical twins (*38*).

Because the embryo has only one chance to allocate these tissue types correctly, it is not surprising that the DV gradient is tightly regulated. The organizer secretes the BMP antagonists Noggin, Follistatin, and Chordin, and if all three are knocked down with morpholino oligonucleotides, the embryo lacks all dorsal structures (*39*). A large network of Chordin-interacting extracellular proteins has been isolated from the *Xenopus* embryo, with key supporting insights coming from zebrafish genetics. Using a combination of biochemistry with purified proteins and embryological studies involving the depletion of multiple gene products with morpholinos and transplantation experiments, it was possible to construct the biochemical pathway shown in Fig. 2E (*40*). All of its components are secreted proteins that are able to directly interact with each other, forming feedback loops of activators and inhibitors synthesized by cells in the dorsal and ventral poles of the embryo.

Chordin, the homolog of Sog, is a morphogen secreted very abundantly by the organizer. It binds to both dorsal (BMP2 and ADMP) and ventral (BMP4/7) BMPs. Tsg, which is expressed ventrally, greatly facilitates the binding of Chordin to BMPs. Studies in zebrafish have shown that heterodimers of BMP2b:BMP7 can activate BMP signaling in the context of the embryo, whereas the respective homodimers do not (*41*). This effect is due to the recruitment of two distinct type I BMP receptors and is markedly similar to *Drosophila* DV patterning in which Sog preferentially binds Dpp:Scw heterodimers, which constitute the most potent signaling ligands. The rate-limiting step in the pathway is the secreted metalloproteinase Tolloid [called Xolloid-related (Xlr) in *Xenopus*] that specifically cleaves Chordin at two particular sites, releasing active BMPs in the ventral side of the embryo (*42*). Tolloid activity is highly regulated. First, it is inhibited by Sizzled, a ventral sFRP (secreted Frizzled-related protein) that functions as a competitive inhibitor of Tolloid, indirectly inhibiting BMP by stabilizing Chordin (*43*). Second, Tolloid protease activity is noncompetitively inhibited by BMPs that directly bind to its noncatalytic CUB domains, explaining the antimorphic (low-BMP) effects of some mutations in *Drosophila* (*44*). Third, the dorsally produced Olfactomedin-related Ont-1 adaptor bridges the binding of Chordin and Tolloid, facilitating Chordin degradation in the dorsal side (*45*). Finally, the Sizzled homolog Crescent acts as an inhibitor of Tolloid on the dorsal side (Fig. 2E). The ventral side produces Crossveinless 2 (CV2, also known as Bmper), an antagonist with BMP-binding domains similar to those of Chordin that does not diffuse and remains on the surface of the cells that produce it. CV2 binds Chordin/BMP with high affinity, concentrating these complexes on the ventral side where they can be cleaved by Tolloid (*46*). The pro-BMP effects of CV2 in the *Drosophila* wing (*47*) may similarly be explained by the concentration of diffusing Dpp/Sog complexes in CV2-expression regions.

For every action in the dorsal side, there is a corresponding reaction in the ventral side. Chordin transcription is activated by high Nodal and low BMP signals. Recent work in zebrafish has shown that microinjection of two different animal pole cells with Nodal and BMP mRNA at the 128-cell stage is sufficient to induce a complete secondary axis (*48*). Self-organization in the *Xenopus* embryo results from the dorsal and ventral genes being under opposite transcriptional control: When BMP levels are lowered, synthesis of dorsal BMPs (ADMP, BMP2) is increased, and at high BMP levels, feedback inhibitors such as Sizzled dampen the signal (and expand the gradient; see below).

Many of the secreted proteins in the feedback loops of the DV pathway react directly with each other as imagined by Turing (*5*). For example, Tolloid and Sizzled constitute a classical activator-inhibitor pair arising from the same cellular source: Xlr activates BMP signaling (indirectly, by degrading Chordin), which promotes Sizzled transcription, and Sizzled protein would in turn diffuse, turning off Tolloid activity in the periphery. The evolutionary conservation of the DV-interacting proteins (such as BMP/Dpp, Chordin/Sog, Tsg, and CV2)—on opposite sides of the embryo—in *Drosophila* and *Xenopus* provides molecular support for the 1822 proposal by French naturalist Etienne Geoffroy Saint-Hilaire that an inversion of the DV body plan has taken place (*49*).

### The Chordin gradient

Using an improved immunolocalization method it has recently become possible to visualize the endogenous Chordin gradient in the *Xenopus* gastrula (*40*). Chordin was found to diffuse within the narrow space that separates the ectoderm from anterior endoderm and mesoderm (Fig. 2, F and G). In amphibian embryos, this virtual cavity is called Brachet's cleft (in honor of the Belgian embryologist). However, all vertebrate embryos have an ECM containing fibronectin and other proteins between the ectoderm and mesoderm. Therefore, Brachet's cleft is not an amphibian-specific structure. Confocal optical sections reveal a smooth gradient of Chordin, extending from the organizer to the ventral side through this ECM (Fig. 2, G, J, and K). Chordin protein diffuses far from the Spemann organizer cells in which it is transcribed (Fig. 2I). The Chordin morphogen gradient extends over a distance of 2 mm (the *Xenopus* gastrula has a diameter of 1.3 mm) in this signaling highway between the ectoderm and

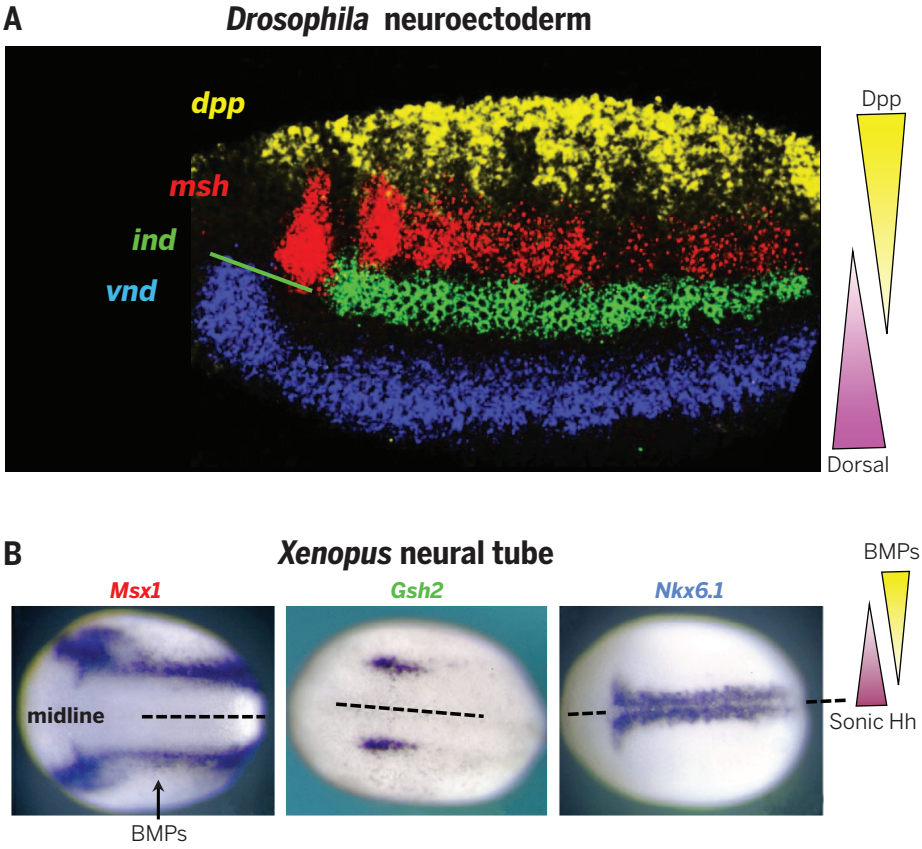



**Fig. 3. BMP-mediated patterning in the CNS of *Drosophila* and vertebrates.** (**A**) Expression patterns of the neural identity genes *vnd* (blue), *ind* (green), and *msh* (red) in a *Drosophila* blastoderm embryo relative to the BMP (*dpp*, yellow) and Dorsal gradients. (**B**) Expression patterns of *Xenopus Msx1* (≈ *msh*), *Gsh2* (≈ *ind*), and Nkx6.1 (≈ vnd) at the open neural plate stage relative to the BMP and Sonic Hedgehog gradients. [Credits: (A) modified figure 1b of (*115*); (B) assembled from panels B (*Gsh2*), K (*Nkx6.1*), and L (*Msx1*) of figure 4 from (*116*)]

endomesoderm (Fig. 2, J and K). The Chordin gradient rescales in bisected embryos, and a second long-distance gradient is seen emanating from Spemann organizer grafts (*40*). Chordin protein must reach very high concentrations in the confines of Brachet's cleft. From this ECM, Chordin protein could pattern both the ectoderm and the mesoderm; for example, causing CNS induction in the overlying ectoderm.

During gastrulation the germ layers undergo extensive morphogenetic movements, and cells might read their positional information directly from the Chordin/BMP gradient contained in the Brachet's cleft ECM. The gradient would be generated by the facilitated diffusion of Chordin from the organizer to regions of lower concentration (carrying with it BMPs made in more dorsal regions) and the sink provided by its degradation by Tolloid in ventral regions. The location of this gradient may explain why the ectoderm and mesoderm respond coordinately to BMPs during development. Diffusion of overexpressed epitope-tagged Nodal and Lefty has also been reported in the ECM flanking both sides of the lateral plate mesoderm in *Xenopus* tailbud tadpoles (*50*), suggesting that diffusion of morphogens through ECM, separating cell layers, might be a more general phenomenon in development. In *Drosophila*, the Sog/Dpp gradient most likely forms in the perivitelline space; at this time, it is unclear whether this extracellular space has any topological homology to Brachet's cleft.

### BMP patterning of the vertebrate dorsal-lateral CNS

After neural induction and involution of the mesoderm, the neural plate invaginates to form the neural tube, which comes to lie between the overlying epidermis and the ventral notochord. BMPs initially produced by epidermal cells diffuse into the dorsal CNS and activate autonomous expression of BMPs within the neural tube. These BMPs then diffuse ventrally to create an activity gradient that has been proposed to activate neural genes (such as Msx1/2) dorsally and to repress genes expressed more ventrally in response to Hedgehog signaling [reviewed in (*51*, *52*)]. A notable parallel between patterning of the vertebrate and *Drosophila* CNS is that orthologs of the *Drosophila* neural identity genes are expressed in the same order relative to the epidermal source of BMPs: *Msx1,2* (= *msh*) dorsally; *Gsh1,2* (= *ind*) laterally, and *Nkx2.2/Nkx6.1* (= Vnd) in the ventral neural tube (Fig. 3, A and B). These relative gene expression domains are also shared with the annelid worm *Platyneris dumerilli* (*53*), suggesting that the CNS of *Urbilateria*, the common ancestor of bilaterians, had at least three subdivisions corresponding to primary rows of neuronal progenitors (*54*). How BMP-mediated regulation of this conserved suite of gene expression may have evolved is discussed further below.

## Quantitative modeling of morphogen gradient formation and activity

The panoply of mechanistic experimental data summarized above has spurred development of increasingly complete and predictive mathematical models of BMP-mediated patterning (*16*, *29*, *55*–*58*). In several instances, these models have suggested potential new network behaviors that have subsequently been tested experimentally and verified. This modeling can help address questions such as how a morphogen gradient leads to reliable patterning and how patterning can be coupled to growth in some cases (*59*). These are nontrivial problems for several reasons. First, it is difficult to imagine mechanisms by which a single morphogen could specify thresholds varying over two or more orders of magnitude in concentration. Second, there is great variability and fluctuation in many cellular functions such as changes in cell shape, size, surface-to-volume ratio, number of cell-surface receptors, protein concentrations based on transcriptional interruption during cell division and transcriptional bursting, and noise that is inherent to all of these and other processes required for cells to measure and respond to a given level of morphogen. Yet, despite these considerable signal-degrading factors, embryos and appendages develop with marked fidelity and are surprisingly resistant to a variety of experimental perturbations (e.g., scaling the overall shape of structures with great accuracy in the face of major alterations in the size of those structures). We consider here several mechanisms for creating and responding to morphogen gradients, as well as homeostatic corrective mechanisms, which, in aggregate, may help account for how such reproducible patterning is achieved.

### Creating morphogen gradients

Although beyond the scope of the current Review, several different mechanisms have been proposed for the creation of stable morphogen gradients (Fig. 4). Perhaps the most obvious and commonly considered mechanism is free diffusion of the secreted morphogen in the extracellular space (Fig. 4A). Models assuming extracellular diffusion of BMPs and other morphogens (e.g., Wnts and Hedgehog-related factors) are consistent with experimental observations in diverse systems. Additionally, there is direct evidence for such a simple mechanism in forming BMP activity gradients in the *Xenopus* gastrula (see above) and the *Drosophila* wing imaginal disc (*60*, *61*), as well as indirect modeling support in other systems (*62*). There is also evidence for other means of morphogen transport (Fig. 4B), including vesicle-bound release from cells (exosomes or argosomes) (*63*, *64*), transcytosis, movement or migration of morphogen-producing cells (*65*), and direct long-distance cell-to-cell contacts mediated by filopodia-like processes called cytonemes (*66*–*71*). Cytonemes can extend, in a directed fashion, more than 100 μm from a cell and can mediate reception of specific morphogen signals [e.g., Dpp versus Hh or FGF (*67*)] (Fig. 4C). In the case of migrating tracheal cells in the *Drosophila* wing imaginal disc, mutations that inhibit the formation of cytonemes abrogate the ability of tracheal cells to respond to Dpp produced in the wing disc epithelium (*66*), strongly suggesting that these cytoplasmic extensions play an essential role in this form of inductive signaling.

Activity gradients of a morphogen can also be created by temporal mechanisms. One class of time-integrating mechanisms is for cells to retain a "memory" of having been exposed to a certain level of morphogen in the past. Such memory can be accomplished directly by perdurance of a morphogen within a cell (e.g., low turnover rate) or indirectly via the activation of a stable switch of some kind. For example, in the case of Hh signaling in the *Drosophila* wing imaginal disc, the width of the Hh responsive domain (six to eight cells) remains constant during the growth of the wing disc, whereas cells at the outer edge of the Hh receptive domain move out of range as the disc grows (*72*). In this situation, if memory of the Hh signal fades on a time scale on the order of the growth rate, then a gradient of Hh response will be observed in cells lying anterior to those currently receiving the diffusible signal. Similarly, in the case of Wg signaling, a tethered form of the ligand can largely replace the function of the normally secreted form in long-range patterning, partly due to the formation of a crude activity gradient that likely reflects memory of contact-mediated signaling and cell displacement during tissue growth (*73*).

Transient patterning events may also seed the outcome of bistable cell fate choices. For example, differences in the genomic length of a locus result in differential temporal delays in gene activation or repression and can lead to the formation of transient spatial gradients (*74*), which in principle could bias the outcome of stable cross-regulatory interactions among those genes. Indeed, the length of target genes of several morphogen gradients (including Dpp) in the *Drosophila* embryo and wing disc follows an ordered trend with respect to the morphogen-defined axes (*74*). Differential delays in the responses of particular genes to a morphogen may also occur [e.g., a DV progression of neural identity gene expression in both *Drosophila* and vertebrates (*75*–*77*)]. Determining the contributions of such diverse spatial and temporal mechanisms for creating gradients of morphogen activity in different developmental settings is one the most important challenges for future studies.

### Responding to morphogen gradients

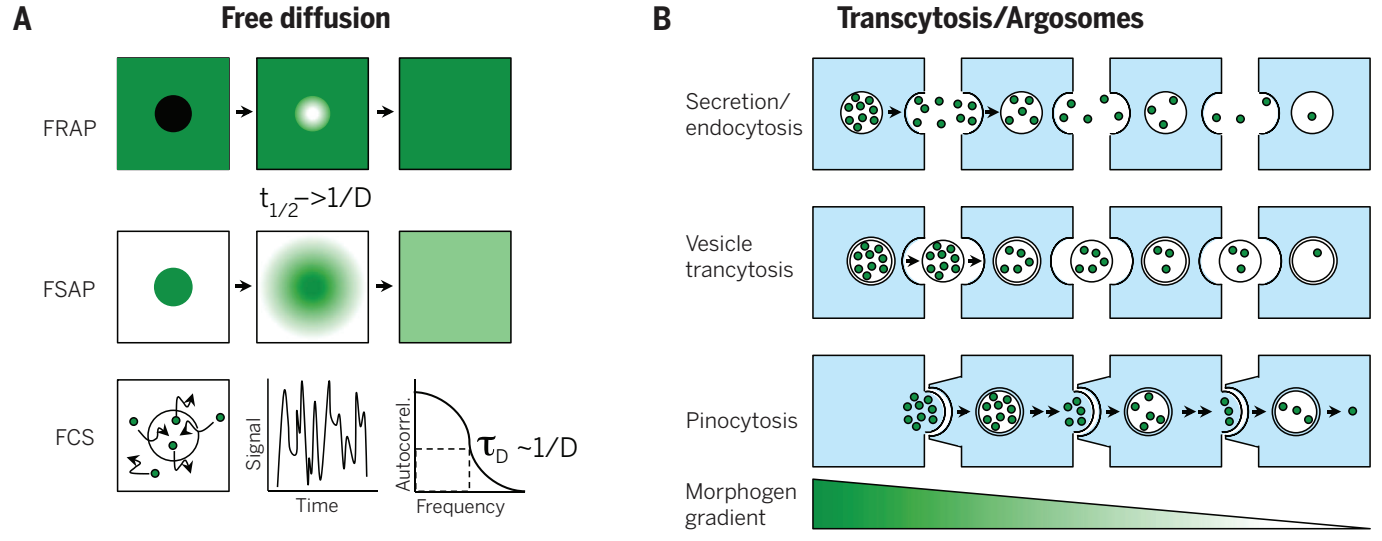
In French flag models, the salient feature of the morphogen gradient read by cells is the absolute level of the morphogen. Mathematical models built around this simple premise have revealed trade-offs in patterning performance regarding parameters such as peak ligand or receptor levels, receptor turnover, receptor occupancy, or ligand diffusion length scale (*58*, *78*). Thus, parameter sets that do well in one part of the gradient typically lead to poor or mediocre performance in other regions. However, by nature a gradient has other features that cells could also detect, including its slope, inflection points, and temporal elements (e.g., the time derivative of a signal or its integrated levels). Different levels of signal-to-noise could also be used, in principle, to estimate

distances far from a source of morphogen because stochastic differences in signaling between neighboring cells should be graded toward the tail end of a gradient. Parallel processing of these various features of a gradient may allow cells to detect their positions across the full expanse of the gradient (Fig. 5A).

### *Homeostatic feedback mechanisms*

It has become increasingly evident that homeostatic feedback mechanisms play a key role in establishing and maintaining reproducible morphogen gradients in the face of challenges such as tissue growth, noise, morphogenetic movements, and variable environmental inputs (e.g., nutrient availability, temperature). Expanders such as Sizzled in *Xenopus* embryos (Fig. 2E) or Pentagone (Fig. 5B) in *Drosophila* wing discs [reviewed in (*14*, *55*)] provide examples of factors that can scale the gradient length constant to the growth of tissues. Expanders, which are typically produced at the low end of a gradient (by virtue of having their expression inhibited by morphogen signaling) and are highly diffusible, bind to ligands and stabilize them [e.g., by preventing their degradation (*79*)]. The stabilizing effect of Pentagone, which may act via its interaction with the glypican Dally (*80*) (a Dpp co-receptor), allows Dpp to travel further as the tissue grows (Fig. 5B), thereby increasing the gradient length constant and scaling the patterning response.

Local interactions between cells can also act homeostatically to integrate growth or environmental



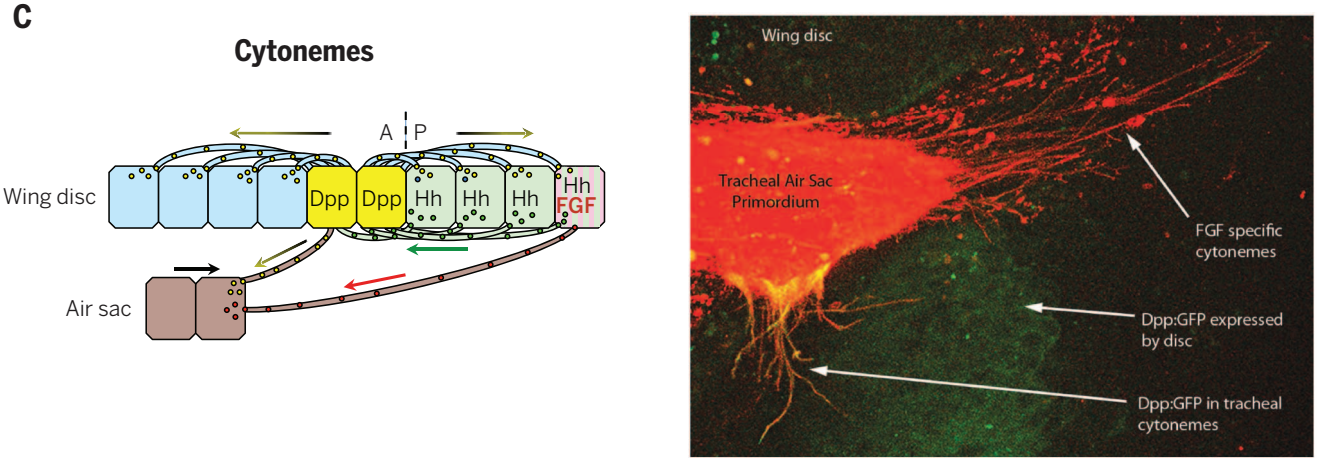




**Fig. 4. Mechanisms of morphogen movement.** (**A**) Methods for measuring free diffusion of a morphogen. Fluorescence recovery after photobleaching (FRAP) provides an estimate for 1/*D* approximately equal to the half time required to regain fluorescence in a region after photobleaching of a fluorophore-tagged morphogen. Fluorescence spread after photoconversion (FSAP) provides a reciprocal estimate of the dispersion rate after activation of a fluorophore-tagged morphogen. Fluorescence correlation spectroscopy (FCS) measures small fluctuations in fluorescence signals within small regions of extracellular space, which, when analyzed, statistically reveal the number of particles moving in and out of that region, thus allowing an estimate of the diffusion constant. For a more in depth treatment, see (*62*). $\tau_D$, diffusion time. (**B**) Diagram summarizing potential mechanisms of active morphogen transport between cells, including secretion from one cell and reuptake via endocytosis by a neighbor, transcytosis of vesicles, and pinocytosis of a protrusion from one cell by a neighboring cell. (**C**) Cytonemes either transport receptor-ligand complexes back to the cell body or export ligands for release and reuptake at a distance from a signal-producing cell. (Left) Diagram summarizing cytoneme-mediated transport of Dpp, Hh, and FGF ligands in the developing *Drosophila* wing primordium. Within the wing disc monolayer, Hh is transported from producing cells in the posterior compartment to six to eight cell diameters into the anterior compartment. Cells in peripheral regions of the disc send cytonemes toward the center of the disc, where they contact Dpp-producing cells and endocytosis Dpp: Receptor complexes and transport them back to the cell body (*117*). (Right) Air sac cells, a migrating outpocketing of the tracheal system, extend independent classes of cytonemes to contact the overlying wing disc to respond to either Dpp or FGF (*66*). [Credits: (A) adapted from content in boxes 5 and 6 of (*62*); (C) left panel modified and assembled from components in figure 1D of (*117*), right panel from (*66*)]

systems with morphogen-mediated patterning to achieve accurate scaling of morphological structures, as in bisected *Xenopus* embryos that develop into normal tadpoles of half size. Signaling systems—such as the planar polarity and Fat/Yorkie pathways, as well as lateral inhibitory interactions (e.g., Notch signaling)—provide such feedback for patterning [reviewed in (*14*, *81*)].

### *Integration of multiple developmental cues*

The Victorian polymath Francis Galton introduced the concept of the "wisdom of crowds or vox populi" by asking a crowd of 800 people attending a country fair to guess the weight of an ox. Astonishingly, the average of the individual guesses differed from the actual weight (1198 pounds) by only 1 pound (<0.1%), with the median guess being off by only 9 pounds (<1%) (*82*). The accuracy of this collective estimation, which has been put forward as an argument in favor of democracy and other forms of plurality (*83*), was predicated on each member of the crowd having an informed but independent basis for guessing, no prior communication between members of the crowd, and a mechanism to collate these guesses to generate a consensus (average or median estimate). Might a similar strategy help to explain the ability of cells to guess their place in a morphogen gradient? This could be the case if cells possess mechanisms to integrate the variety of potential parallel-acting mechanisms for generating a gradient (e.g., facilitated diffusion, exosomes, cytonemes), each of which could be read independently by distinct compartmentalized receptor complexes, as well as the diversity of gradient information that could impinge on each particular readout of the gradient (e.g., the magnitude, integral, and derivative of the signal) and homeostatic network interactions (e.g., cross-regulation between genes receiving distinct sets of gradient inputs, as well as proportional, integrated, and derivative feedback compensation). Such a consensus-based estimate of position in a morphogen gradient might perform well in establishing the relative position of a cell and, in conjunction with local regulation of secondary feedback signals (e.g., planar signals,

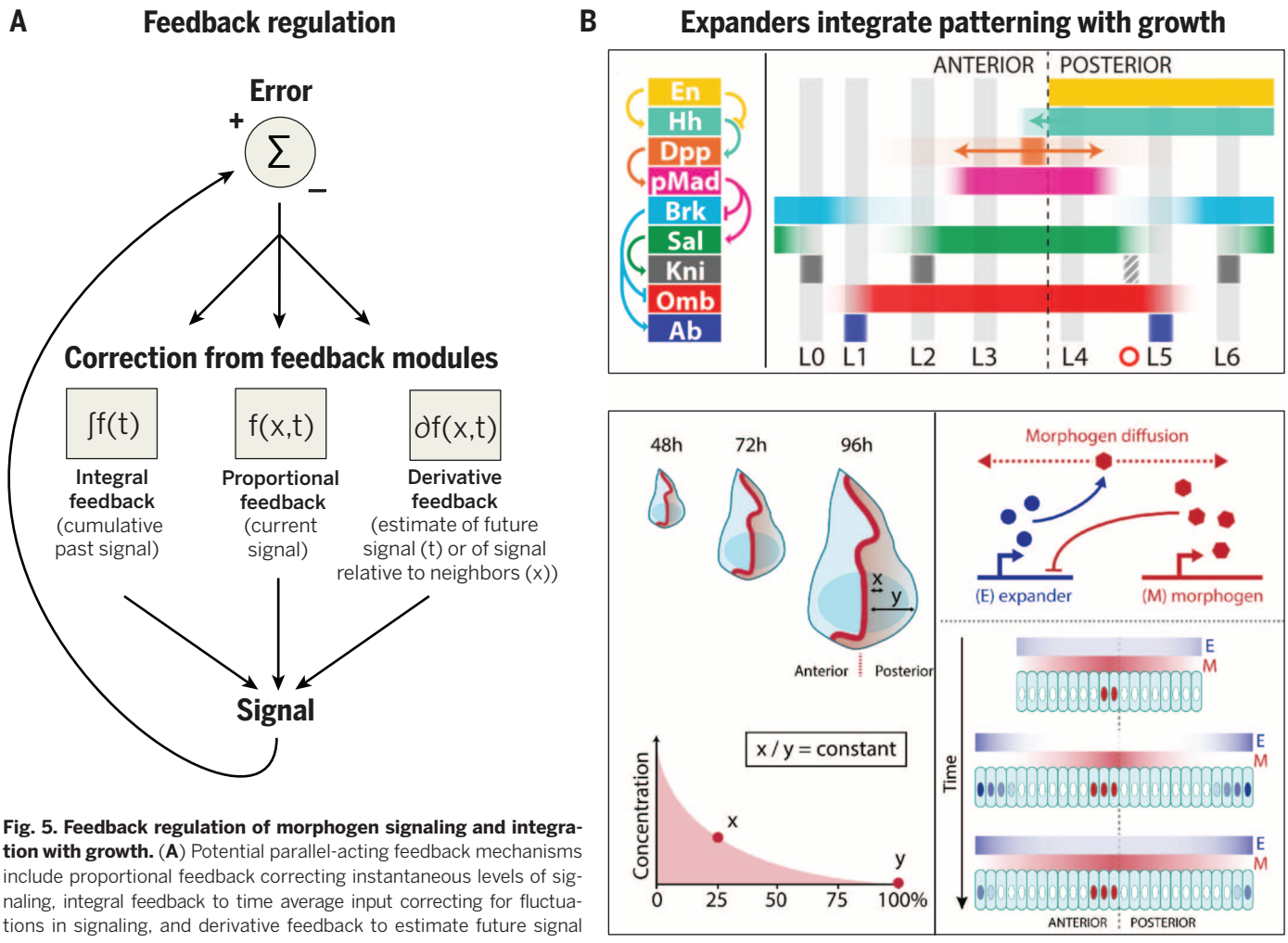



**Fig. 5. Feedback regulation of morphogen signaling and integration with growth.** (**A**) Potential parallel-acting feedback mechanisms include proportional feedback correcting instantaneous levels of signaling, integral feedback to time average input correcting for fluctuations in signaling, and derivative feedback to estimate future signal strength and to integrate signaling locally in space. (**B**) Integration of patterning and growth. (Top) Scheme diagramming the gene regulatory network (left) controlling BMP-mediated anterior-posterior patterning in the wing imaginal disc (right). Briefly, the Engrailed (En) transcription factor activates Hh expression in the posterior compartment. Hh then diffuses anteriorly to activate Dpp expression in a central stripe. Dpp diffuses both anteriorly and posteriorly to activate genes such as *spalt* (*sal*) and *omb* in a threshold-dependent fashion. Wing vein primordia (numbered L1 to L6) are then induced along specific gene expression boundaries (e.g., the L2 vein anterior to the *sal* expression domain and the L5 vein along the posterior *omb* border) (*59*, *61*, *118*). (Lower Left) BMP-mediated patterning in the growing wing imaginal disc (≈1000-fold increase in cell number). The disc increases in size, whereas relative positions of gene expression patterns remain constant (i.e., *X*/*Y* = constant), indicating that the length constant of the Dpp gradient increases as the disc expands. (Lower Right) The expander Pentagone contributes to scaling in the wing disc. Pentagone expression (in blue) is repressed by Dpp (in red) signaling, but it protects Dpp from receptor-mediated endocytosis, thereby allowing it to diffuse a greater distance. As the disc grows, Dpp-mediated inhibition weakens at the periphery of the disc, allowing expression of Pentagone, which in turn facilitates diffusion of Dpp. [Credits: (B) drawn and provided by Valentino Gantz]

lateral inhibitory factors, expanders), may iteratively provide the necessary corrections to achieve proper scaling. Identifying cellular mechanisms for integrating such hypothetical consensus-based integration of diverse gradient estimates and determining whether cells in different developmental contexts employ distinct guessing algorithms (e.g., does inductive cell signaling rely more on cytonemes, whereas long-range patterning depends primarily on facilitated diffusion?) will be important steps in assessing the validity of this hypothesis.

## Evolution and diversification of DV patterning systems

As mentioned earlier, the role of polarized BMP signaling in establishing the DV axis is one the best examples for evolutionary conservation of a developmental patterning system. For example, injection of *Drosophila sog* mRNA into ventral regions of a *Xenopus* embryo lead to axis duplications similar to those observed with injection of chordin mRNA or transplantation of the Spemann organizer (*84*, *85*). Similarly, vertebrate BMP pathway components are active in *Drosophila* and, in the case of BMP2, can even rescue *dpp*-null mutants to full viability (*86*). This high degree of functional conservation, in combination with the similar relative expression patterns of pathway components in organisms spanning a broad range of phyla, provides one of the best examples of a conserved developmental system.

### Ancestral role of BMP-mediated axial patterning

Studies across a broad spectrum of organisms have provided further evidence for the conserved role of BMP signaling in DV patterning and subdivision of the embryo into neural versus epidermal domains (i.e., neural induction in the broadest sense). Thus, BMPs and their antagonists define epidermal versus neural cell fates in arthropods [e.g., basal insects (*87*) and spiders (*88*)], lophotrochozoa [e.g., planaria (*89*–*91*) and polychaete annelids (*53*)], and deuterostomes [echinoderms (*91*) and nonvertebrate chordates; e.g., amphioxus (*93*)] [reviewed in (*4*, *94*)].

BMPs and their antagonists are also expressed in localized patterns in diploblast embryos (i.e., cnidarians, the sister group to bilateria, comprising jellyfish, sea anemones, corals, and hydra) where they play an important role in establishing primary body axes (*95*, *96*). As diploblasts have diffuse nerve nets, the presence of polarized BMP signaling in these species suggests that axial patterning by BMPs preceded centralization of the nervous system. During gastrulation, the *Nematostella* (sea anemone) gastrula embryo forms a directive axis that expresses *Chordin, Dpp*, and *BMP5-8* on one side and the BMP *GDF-like* and the BMP antagonist *Gremlin* on the opposite side. Although Chordin and Dpp are secreted by the same group of cells (Fig. 6), signaling by phospho-Smad1/5 takes place in the opposite side, where likely Dpp:BMP5-8 dimers are liberated from Chordin inhibition by Tolloid (*95*). This ancestral long-distance signaling pathway has marked similarities to the ones present in *Xenopus*, zebrafish, and *Drosophila*, except that in *Nematostella* this gradient also controls the expression of *Hox* genes.

A variety of evidence suggests that the role for BMPs in specifying epidermis and a condensed nervous system arose in an uribilaterian ancestor. First, as mentioned above, BMPs perform these two functions in diverse organisms spanning all three major bilaterian branches. Second, neural identity genes are expressed in a conserved series of DV domains in much the same fashion that *Hox* genes are expressed along the AP axis. Third, species with condensed CNS organization are present within the great majority of the 30 bilaterian phyla (*97*). Many phyla also contain organisms with simpler body designs, which are likely to have arisen secondarily as derived simplifications of the basal body plan. For example, in hemichordates, which have a diffuse

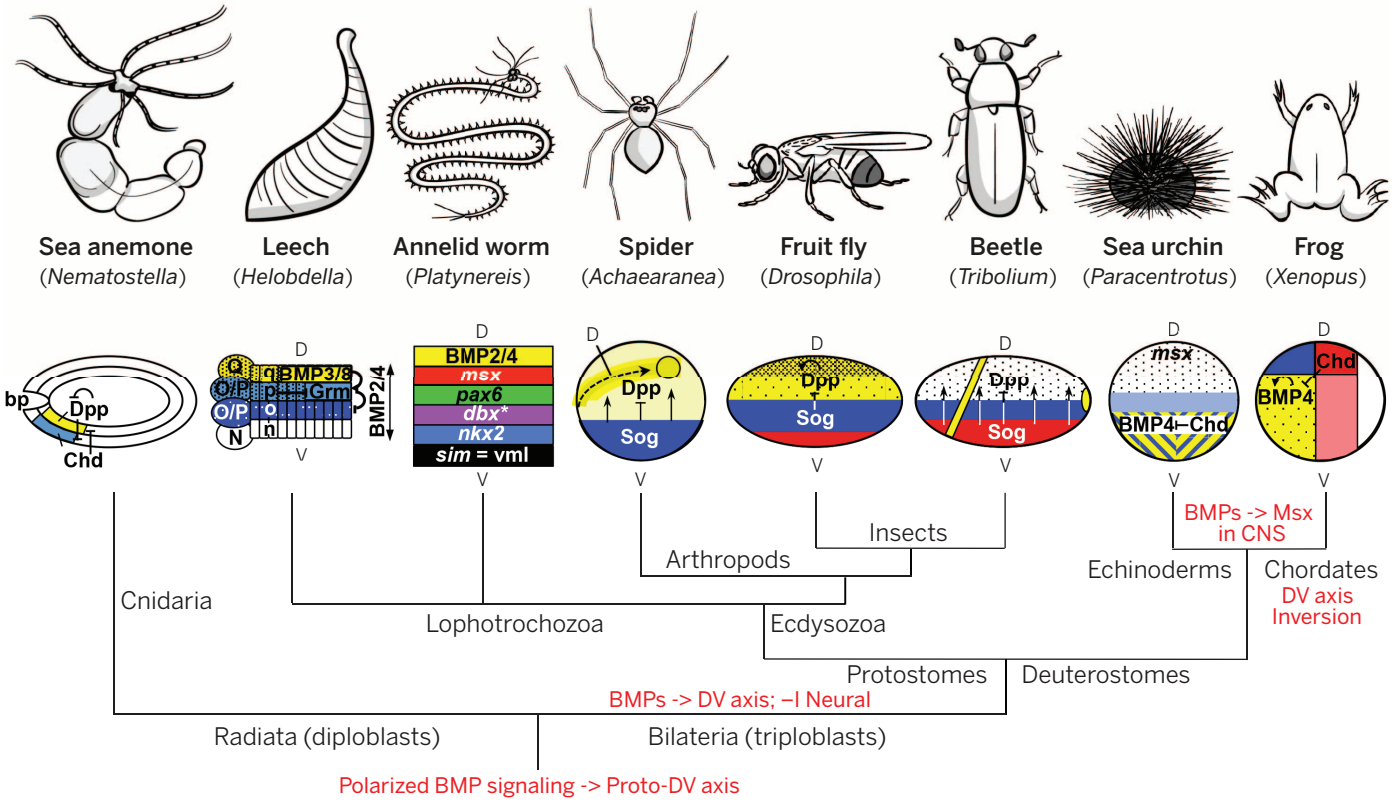



**Fig. 6. Evo-devo analysis of BMP-mediated patterning of the DV axis.** Phylogeny of select organisms in which BMP signaling has been studied is shown. Despite many alterations in expression patterns of BMPs and their antagonists, the developmental outcome of this signaling pathway has remained conserved across these diverse organisms (e.g., BMP signaling suppresses neural fates). Red text indicates nodes at which specific changes in BMP signaling may have occurred. [Credits: Evolutionary diagram modified and expanded from figure 1 of (*94*). Sketches of organisms drawn and provided by Valentino Gantz]

nerve network, suppression of neuronal development by the BMP gradient has been lost. However, the components of this pathway are still expressed in a polarized pattern, which suggests that they are required for other DV patterning functions such as determining the ventral position of the mouth (*98*).

### Evolvability of BMP-mediated patterning

Although, as argued above, it seems likely that BMP-mediated axial patterning is an ancestral trait, there is also a marked degree of flexibility in how particular BMP pathway components are deployed to achieve the common goal of generating epidermis in regions with high levels of BMP signaling and neuroectoderm in regions of low signaling (Fig. 6). For example, in more primitive insects (e.g., beetles), the source of BMPs is not localized along the DV axis, but ventrally produced Sog helps BMPs diffuse to the dorsal side of the embryo (*99*) as it also does in *Drosophila* (*21*, *24*, *29*, *57*, *100*–*106*). Also, in more primitive dipterans such as the scuttle fly, the BMP gradient is broken into two peaks that define distinct tissues in this species, the amnion versus the serosa, in contrast to *Drosophila* with single blended extra-embryonic tissue, the amnioserosa (*107*). In spiders, a cluster of Dpp-producing mesenchymal migratory cells leaves a trail of signaling that specifies the future dorsal midline that may be contacted by epithelial cells via cytonemes (*108*). In the mouse, *chordin* knockout causes only minor phenotypes in the prechordal midline, but in combination with *noggin* knockouts, the forebrain fails to develop (*109*). Thus, in mammals, which have a slower development and lack epiboly movements over a yolk mass (which requires the maintenance of a constant gradient), redundant BMP antagonists increase in developmental importance. In sea urchin embryos, BMPs and Chordin (Chd/Sog) are coexpressed (orally) (as is the case for ADMP, BMP2, and Chordin in *Xenopus*), and the BMP activity gradient forms as a consequence of differences in BMP and Chordin diffusion (*92*). This strategy is analogous to that of sea anemone embryos in which Dpp and Chordin are expressed in the same cells and the key regulator of the system is the shuttling of Chordin (*95*). However, some animals such as annelids seem to have lost *chordin* from their genome, and other BMP antagonists such as Noggin and Gremlin are brought into play (*110*, *111*). In the case of the leech, DV patterning is regulated by BMPs inducing only immediately adjacent cells and up-regulating the BMP antagonist Gremlin (*94*, *111*). These evolutionary developmental biology ("evo-devo") studies have revealed a high degree of evolvability in the ancestral Sog/Chordin/BMP DV patterning gradient.

There is also evidence for evolvability of BMP signaling in CNS patterning. Although BMP activity gradients are consistently oriented with respect to the epidermis and subdomains of the CNS in diverse organisms, it appears that they achieve this conserved output by alternative mechanisms. As summarized above, BMPs act during CNS patterning in *Drosophila* as they do earlier in neural induction to repress expression of neural genes, whereas in vertebrates the consensus view has been that BMPs activate genes in dorsal and lateral regions of the spinal cord (*51*). Analysis of cis-regulatory elements responsible for BMP-mediated regulation of the paralogous *Drosophila msh* and zebrafish *msxB* genes in the dorsal CNS supports opposite modes of BMP regulation (*37*). Mutation of BMP-responsive SMAD sites leads to derepression of *msh* reporter gene expression in the epidermis in *Drosophila*, whereas a comparable mutation results in the loss of *msxB* reporter gene expression in zebrafish. Thus, BMPs can act by opposite mechanisms (weak repression versus weak activation) to achieve the same gene expression output pattern.

## Conclusions and future perspectives

Over the past two decades, the molecular basis for classic embryological observations has been elucidated in great detail and has led to testable quantitative network models for BMP-mediated regulation of DV patterning. This progress notwithstanding, several important questions remain. Perhaps foremost among them is how network models can account for the nearly invariant morphologies of fully developed organisms and how such reproducible patterning is achieved in the face of considerable difficulties in accurately reading morphogen activity across the broad range of graded concentrations, which engenders unavoidable tradeoffs. Such models must also cope with inherent variations in critical parameters arising from both intrinsic noise and environmental variation, which can result in substantial perturbations, as illustrated by embryos of vastly different sizes or developing at different temperatures, forming correctly proportioned adults. It will be interesting to see whether models based on the "wisdom of crowds" concept shed light on this problem. Experimental tests of such models should account for various forms of potential parallel genetic circuitry (or redundancy) that are integral to this line of thinking. Thus, the loss of single or even multiple circuits may not have a major effect on morphology, but the roles of these circuits might be revealed in sensitized backgrounds where other critical elements are weakened. A salient example of such redundancy is the ability of a membrane-tethered form of Wg alone to sustain viability and generate almost normal patterning (*73*), whereas it is nearly certain that diffusible forms of the protein also normally play a role in patterning. Additionally, new quantitative approaches integrated with cutting-edge imaging methods should be considered, such as using Bayesian statistical models to reveal potential links in gene regulatory networks by examining the effects of many (hundreds) modest perturbations of the system (e.g., heterozygosity or duplication of each gene in the network) on multiple gene expression markers in vivo across fields of developing cells [e.g., (*112*, *113*)].

Other important questions include how different stable BMP signaling networks are deployed within an organism to accomplish distinct patterning events and how interactions within these networks can change during evolution while retaining similar developmental outputs. With regard to adaptation of BMP signaling networks to different developmental contexts, an intriguing question is whether different modes of BMP transport dominate in particular settings. For example, might free diffusion facilitated by a sink be a dominant mechanism for dispersing ligands between cell sheets, whereas cytonemes offer a preferred mode of transport for other types of inductive signaling (e.g., during Dpp-dependent induction of the *Drosophila* midgut or in maintenance of local stem niches)? Mutants selectively inhibiting the formation of cytonemes dedicated to specific signaling pathways will provide important new insights into this mode of ligand transport and reception.

Comparative studies of BMP regulatory networks (such as that involved in early embryonic DV patterning and neural induction) in additional species would also be of interest, as well as further analysis of BMP-dependent CNS patterning, as discussed above. An interesting question in this regard is whether critical changes in a network are accomplished at the level of alterations in cis-regulatory elements that shift patterns of gene expression or by the addition or subtraction of specific proteins from the system, such as expanders or other factors mediating feedback interactions. Clearly, many interesting and important questions remain in this paradigm-setting field.

## REFERENCES AND NOTES


1. V. Hamburger, *The Heritage of Experimental Embryology: Hans Spemann and the Organizer* (Oxford Univ. Press, Oxford, 1988).
2. H. M. Spemann, H. Mangold, Uber induction von embryonanlagen durch implantation artfremder organis atoren. *W. Roux' Arch. Ent. Org.* **100**, 599–638 (1924) [translated and reprinted in *Int. J. Dev. Biol.* **45**, 15 (2001)].
3. E. M. De Robertis, Evo-devo: Variations on ancestral themes. *Cell* **132**, 185–195 (2008). doi: 10.1016/j.cell.2008.01.003; pmid: 18243095
4. C. M. Mizutani, E. Bier, EvoD/Vo: The origins of BMP signalling in the neuroectoderm. *Nat. Rev. Genet.* **9**, 663–677 (2008). doi: 10.1038/nrg2417; pmid: 18679435
5. A. Turing, The chemical basis of morphogenesis. *Philos. Trans. R. Soc. London Ser. B* **237**, 37–72 (1952). doi: 10.1098/rstb.1952.0012
6. F. Crick, Diffusion in embryogenesis. *Nature* **225**, 420–422 (1970). doi: 10.1038/225420a0; pmid: 5411117
7. A. Gierer, H. Meinhardt, A theory of biological pattern formation. *Kybernetik* **12**, 30–39 (1972). doi: 10.1007/BF00289234; pmid: 4663624
8. L. Wolpert, Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25**, 1–47 (1969). doi: 10.1016/S0022-5193(69)80016-0; pmid: 4390734
9. P. W. Holland, Evolution of homeobox genes. *WIREs Dev. Biol.* **2**, 31–45 (2013). doi: 10.1002/wdev.78
10. S. J. Gould, *The Structure of Evolutionary Theory* (Harvard Univ. Press, Cambridge, MA, 2002).
11. A. Stathopoulos, M. Levine, Dorsal gradient networks in the *Drosophila* embryo. *Dev. Biol.* **246**, 57–67 (2002). doi: 10.1006/dbio.2002.0652; pmid: 12027434
12. S. Ross, C. S. Hill, How the Smads regulate transcription. *Int. J. Biochem. Cell Biol.* **40**, 383–408 (2008). doi: 10.1016/j.biocel.2007.09.006; pmid: 18061509
13. A. Weiss *et al.*, A conserved activation element in BMP signaling during *Drosophila* development. *Nat. Struct. Mol. Biol.* **17**, 69–76 (2010). doi: 10.1038/nsmb.1715; pmid: 20010841
14. F. Hamaratoglu, M. Affolter, G. Pyrowolakis, Dpp/BMP signaling in flies: From molecules to biology. *Semin. Cell Dev. Biol.* **32**, 128–136 (2014). doi: 10.1016/j.semcdb.2014.04.036; pmid: 24813173

15. H. Araujo, M. R. Fontenele, R. N. da Fonseca, Position matters: Variability in the spatial pattern of BMP modulators generates functional diversity. *Genesis* **49**, 698–718 (2011). doi: 10.1002/dvg.20778; pmid: 21671348
16. L. Zakin, E. M. De Robertis, Extracellular regulation of BMP signaling. *Curr. Biol.* **20**, R89–R92 (2010). doi: 10.1016/j.cub.2009.11.021; pmid: 20144774
17. D. Umulis, M. B. O'Connor, S. S. Blair, The extracellular regulation of bone morphogenetic protein signaling. *Development* **136**, 3715–3728 (2009). doi: 10.1242/dev.031534; pmid: 19855014
18. V. Francois, M. Solloway, J. W. O'Neill, J. Emery, E. Bier, Dorsal-ventral patterning of the *Drosophila* embryo depends on a putative negative growth factor encoded by the *short gastrulation* gene. *Genes Dev.* **8**, 2602–2616 (1994). doi: 10.1101/gad.8.21.2602; pmid: 7958919
19. B. Biehs, V. François, E. Bier, The *Drosophila short gastrulation* gene prevents Dpp from autoactivating and suppressing neurogenesis in the neuroectoderm. *Genes Dev.* **10**, 2922–2934 (1996). doi: 10.1101/gad.10.22.2922; pmid: 8918893
20. A. Jaźwińska, C. Rushlow, S. Roth, The role of brinker in mediating the graded response to Dpp in early *Drosophila* embryos. *Development* **126**, 3323–3334 (1999). pmid: 10393112
21. O. Shimmi, D. Umulis, H. Othmer, M. B. O'Connor, Facilitated transport of a Dpp/Scw heterodimer by Sog/Tsg leads to robust patterning of the *Drosophila* blastoderm embryo. *Cell* **120**, 873–886 (2005). doi: 10.1016/j.cell.2005.02.009; pmid: 15797386
22. S. Srinivasan, K. E. Rashka, E. Bier, Creation of a Sog morphogen gradient in the *Drosophila* embryo. *Dev. Cell* **2**, 91–101 (2002). doi: 10.1016/S1534-5807(01)00097-1; pmid: 11782317
23. G. Marqués *et al*., Production of a DPP activity gradient in the early *Drosophila* embryo through the opposing actions of the SOG and TLD proteins. *Cell* **91**, 417–426 (1997). doi: 10.1016/S0092-8674(00)80425-0; pmid: 9363950
24. Y. C. Wang, E. L. Ferguson, Spatial bistability of Dpp-receptor interactions during *Drosophila* dorsal-ventral patterning. *Nature* **434**, 229–234 (2005). doi: 10.1038/nature03318; pmid: 15759004
25. E. Negreiros, M. Fontenele, A. R. Câmara, H. Araujo, αPS1βPS integrin receptors regulate the differential distribution of Sog fragments in polarized epithelia. *Genesis* **48**, 31–43 (2010). pmid: 20017203
26. H. Araujo, E. Negreiros, E. Bier, Integrins modulate Sog activity in the *Drosophila* wing. *Development* **130**, 3851–3864 (2003). doi: 10.1242/dev.00613; pmid: 12835400
27. A. Sawala, C. Sutcliffe, H. L. Ashe, Multistep molecular mechanism for bone morphogenetic protein extracellular transport in the *Drosophila* embryo. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 11222–11227 (2012). doi: 10.1073/pnas.1202781109; pmid: 22733779
28. A. Aleman *et al*., Mad linker phosphorylations control the intensity and range of the BMP-activity gradient in developing *Drosophila* tissues. *Sci. Rep.* **4**, 6927 (2014). doi: 10.1038/srep06927; pmid: 25377173
29. D. M. Umulis, O. Shimmi, M. B. O'Connor, H. G. Othmer, Organism-scale modeling of early *Drosophila* patterning via bone morphogenetic proteins. *Dev. Cell* **18**, 260–274 (2010). doi: 10.1016/j.devcel.2010.01.006; pmid: 20159596
30. J. Gavin-Smyth, Y. C. Wang, I. Butler, E. L. Ferguson, A genetic network conferring canalization to a bistable patterning system in *Drosophila*. *Curr. Biol.* **23**, 2296–2302 (2013). doi: 10.1016/j.cub.2013.09.055; pmid: 24184102
31. H. L. Liang, M. Xu, Y. C. Chuang, C. Rushlow, Response to the BMP gradient requires highly combinatorial inputs from multiple patterning systems in the *Drosophila* embryo. *Development* **139**, 1956–1964 (2012). doi: 10.1242/dev.079772; pmid: 22513375
32. K. Yu *et al*., Processing of the *Drosophila* Sog protein creates a novel BMP inhibitory activity. *Development* **127**, 2143–2154 (2000). pmid: 10769238
33. K. Carneiro *et al*., Graded maternal short gastrulation protein contributes to embryonic dorsal-ventral patterning by delayed induction. *Dev. Biol.* **296**, 203–218 (2006). doi: 10.1016/j.ydbio.2006.04.453; pmid: 16781701
34. K.-H. Kang, E. Bier, dHIP14-dependent palmitoylation promotes secretion of the BMP antagonist Sog. *Dev. Biol.* **346**, 1–10 (2010). doi: 10.1016/j.ydbio.2010.06.024; pmid: 20599894
35. C. M. Mizutani, N. Meyer, H. Roelink, E. Bier, Threshold-dependent BMP-mediated repression: A model for a conserved mechanism that patterns the neuroectoderm. *PLOS Biol.* **4**, e313 (2006). doi: 10.1371/journal.pbio.0040313; pmid: 16968133
36. J. Cowden, M. Levine, Ventral dominance governs sequential patterns of gene expression across the dorsal-ventral axis of the neuroectoderm in the *Drosophila* embryo. *Dev. Biol.* **262**, 335–349 (2003). doi: 10.1016/S0012-1606(03)00395-6; pmid: 14550796
37. F. F. Esteves *et al*., BMPs regulate *msx* gene expression in the dorsal neuroectoderm of *Drosophila* and vertebrates by distinct mechanisms. *PLOS Genet.* **10**, e1004625 (2014). doi: 10.1371/journal.pgen.1004625; pmid: 25210771
38. E. M. De Robertis, H. Kuroda, Dorsal-ventral patterning and neural induction in *Xenopus* embryos. *Annu. Rev. Cell Dev. Biol.* **20**, 285–308 (2004). doi: 10.1146/annurev.cellbio.20.011403.154124; pmid: 15473842
39. M. K. Khokha, J. Yeh, T. C. Grammer, R. M. Harland, Depletion of three BMP antagonists from Spemann's organizer leads to a catastrophic loss of dorsal structures. *Dev. Cell* **8**, 401–411 (2005). doi: 10.1016/j.devcel.2005.01.013; pmid: 15737935
40. J. L. Plouhinec, L. Zakin, Y. Moriyama, E. M. De Robertis, Chordin forms a self-organizing morphogen gradient in the extracellular space between ectoderm and mesoderm in the *Xenopus* embryo. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 20372–20379 (2013). doi: 10.1073/pnas.1319745110; pmid: 24284174
41. S. C. Little, M. C. Mullins, Bone morphogenetic protein heterodimers assemble heteromeric type I receptor complexes to pattern the dorsoventral axis. *Nat. Cell Biol.* **11**, 637–643 (2009). doi: 10.1038/ncb1870; pmid: 19377468
42. S. Piccolo *et al*., Cleavage of Chordin by Xolloid metalloprotease suggests a role for proteolytic processing in the regulation of Spemann organizer activity. *Cell* **91**, 407–416 (1997). doi: 10.1016/S0092-8674(00)80424-9; pmid: 9363949
43. H. X. Lee, A. L. Ambrosio, B. Reversade, E. M. De Robertis, Embryonic dorsal-ventral signaling: Secreted frizzled-related proteins as inhibitors of tolloid proteinases. *Cell* **124**, 147–159 (2006). doi: 10.1016/j.cell.2005.12.018; pmid: 16413488
44. H. X. Lee, F. A. Mendes, J. L. Plouhinec, E. M. De Robertis, Enzymatic regulation of pattern: BMP4 binds CUB domains of Tolloids and inhibits proteinase activity. *Genes Dev.* **23**, 2551–2562 (2009). doi: 10.1101/gad.1839309; pmid: 19884260
45. H. Inomata, T. Haraguchi, Y. Sasai, Robust stability of the embryonic axial pattern requires a secreted scaffold for chordin degradation. *Cell* **134**, 854–865 (2008). doi: 10.1016/j.cell.2008.07.008; pmid: 18775317
46. A. L. Ambrosio *et al*., Crossveinless-2 is a BMP feedback inhibitor that binds Chordin/BMP to regulate *Xenopus* embryonic patterning. *Dev. Cell* **15**, 248–260 (2008). doi: 10.1016/j.devcel.2008.06.013; pmid: 18694564
47. A. Ralston, S. S. Blair, Long-range Dpp signaling is regulated to restrict BMP signaling to a crossvein competent zone. *Dev. Biol.* **280**, 187–200 (2005). doi: 10.1016/j.ydbio.2005.01.018; pmid: 15766758
48. P. F. Xu, N. Houssin, K. F. Ferri-Lagneau, B. Thisse, C. Thisse, Construction of a vertebrate embryo from two opposing morphogen gradients. *Science* **344**, 87–89 (2014). doi: 10.1126/science.1248252; pmid: 24700857
49. T. A. Appel, *The Cuvier-Geoffroy Debate: French Biology in the Decades Before Darwin* (Oxford Univ. Press, Oxford, 1987).
50. L. Marjoram, C. Wright, Rapid differential transport of Nodal and Lefty on sulfated proteoglycan-rich extracellular matrix regulates left-right asymmetry in *Xenopus*. *Development* **138**, 475–485 (2011). doi: 10.1242/dev.056010; pmid: 21205792
51. K. J. Lee, T. M. Jessell, The specification of dorsal cell fates in the vertebrate central nervous system. *Annu. Rev. Neurosci.* **22**, 261–294 (1999). doi: 10.1146/annurev.neuro.22.1.261; pmid: 10202540
52. I. Patten, M. Placzek, Opponent activities of Shh and BMP signaling during floor plate induction in vivo. *Curr. Biol.* **12**, 47–52 (2002). doi: 10.1016/S0960-9822(01)00631-5; pmid: 11790302
53. A. S. Denes *et al*., Molecular architecture of annelid nerve cord supports common origin of nervous system centralization in bilateria. *Cell* **129**, 277–288 (2007). doi: 10.1016/j.cell.2007.02.040; pmid: 17448990
54. D. Arendt, A. S. Denes, G. Jékely, K. Tessmar-Raible, The evolution of nervous system centralization. *Philos. Trans. R. Soc. London Ser. B* **363**, 1523–1528 (2008). doi: 10.1098/rstb.2007.2242; pmid: 18192182
55. D. Ben-Zvi, A. Fainsod, B. Z. Shilo, N. Barkai, Scaling of dorsal-ventral patterning in the *Xenopus* laevis embryo. *BioEssays* **36**, 151–156 (2014). doi: 10.1002/bies.201300136; pmid: 24323952
56. Y. T. Zhang, A. D. Lander, Q. Nie, Computational analysis of BMP gradients in dorsal-ventral patterning of the zebrafish embryo. *J. Theor. Biol.* **248**, 579–589 (2007). doi: 10.1016/j.jtbi.2007.05.026; pmid: 17673236
57. C. M. Mizutani *et al*., Formation of the BMP activity gradient in the *Drosophila* embryo. *Dev. Cell* **8**, 915–924 (2005). doi: 10.1016/j.devcel.2005.04.009; pmid: 15935780
58. A. D. Lander, Pattern, growth, and control. *Cell* **144**, 955–969 (2011). doi: 10.1016/j.cell.2011.03.009; pmid: 21414486
59. S. Restrepo, J. J. Zartman, K. Basler, Coordination of patterning and growth by the morphogen DPP. *Curr. Biol.* **24**, R245–R255 (2014). doi: 10.1016/j.cub.2014.01.055; pmid: 24650915
60. D. Nellen, R. Burke, G. Struhl, K. Basler, Direct and long-range action of a DPP morphogen gradient. *Cell* **85**, 357–368 (1996). doi: 10.1016/S0092-8674(00)81114-9; pmid: 8616891
61. S. Zhou *et al*., Free extracellular diffusion creates the Dpp morphogen gradient of the *Drosophila* wing disc. *Curr. Biol.* **22**, 668–675 (2012). doi: 10.1016/j.cub.2012.02.065; pmid: 22445299
62. P. Müller, K. W. Rogers, S. R. Yu, M. Brand, A. F. Schier, Morphogen transport. *Development* **140**, 1621–1638 (2013). doi: 10.1242/dev.083519; pmid: 23533171
63. J. L. Erickson, Formation and maintenance of morphogen gradients: An essential role for the endomembrane system in *Drosophila melanogaster* wing development. *Fly* **5**, 266–271 (2011). doi: 10.4161/fly.5.3.16542; pmid: 21654212
64. V. Greco, M. Hannus, S. Eaton, Argosomes: A potential vehicle for the spread of morphogens through epithelia. *Cell* **106**, 633–645 (2001). doi: 10.1016/S0092-8674(01)00484-6; pmid: 11551510
65. S. Pfeiffer, C. Alexandre, M. Calleja, J. P. Vincent, The progeny of wingless-expressing cells deliver the signal at a distance in *Drosophila* embryos. *Curr. Biol.* **10**, 321–324 (2000). doi: 10.1016/S0960-9822(00)00381-X; pmid: 10744976
66. S. Roy, H. Huang, S. Liu, T. B. Kornberg, Cytoneme-mediated contact-dependent transport of the *Drosophila* decapentaplegic signaling protein. *Science* **343**, 1244624 (2014). doi: 10.1126/science.1244624; pmid: 24385607
67. S. Roy, F. Hsiung, T. B. Kornberg, Specificity of *Drosophila* cytonemes for distinct signaling pathways. *Science* **332**, 354–358 (2011). doi: 10.1126/science.1198949; pmid: 21493861
68. F. Hsiung, F.-A. Ramirez-Weber, D. D. Iwaki, T. B. Kornberg, Dependence of *Drosophila* wing imaginal disc cytonemes on Decapentaplegic. *Nature* **437**, 560–563 (2005). doi: 10.1038/nature03951; pmid: 16177792
69. M. Sato, T. B. Kornberg, FGF is an essential mitogen and chemoattractant for the air sacs of the *Drosophila* tracheal system. *Dev. Cell* **3**, 195–207 (2002). doi: 10.1016/S1534-5807(02)00202-2; pmid: 12194851
70. F. A. Ramírez-Weber, T. B. Kornberg, Cytonemes: Cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell* **97**, 599–607 (1999). doi: 10.1016/S0092-8674(00)80771-0; pmid: 10367889
71. T. A. Sanders, E. Llagostera, M. Barna, Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. *Nature* **497**, 628–632 (2013). doi: 10.1038/nature12157; pmid: 23624372
72. M. Nahmad, A. Stathopoulos, Dynamic interpretation of hedgehog signaling in the *Drosophila* wing disc. *PLOS Biol.* **7**, e1000202 (2009). doi: 10.1371/journal.pbio.1000202; pmid: 19787036
73. C. Alexandre, A. Baena-Lopez, J. P. Vincent, Patterning and growth control by membrane-tethered Wingless. *Nature* **505**, 180–185 (2014). doi: 10.1038/nature12879; pmid: 24390349
74. P. McHale *et al*., Gene length may contribute to graded transcriptional responses in the *Drosophila* embryo. *Dev. Biol.* **360**, 230–240 (2011). doi: 10.1016/j.ydbio.2011.08.016; pmid: 21920356
75. E. Kutejova, J. Briscoe, A. Kicheva, Temporal dynamics of patterning by morphogen gradients. *Curr. Opin. Genet. Dev.* **19**, 315–322 (2009). doi: 10.1016/j.gde.2009.05.004; pmid: 19596567
76. K. F. Liem Jr., G. Tremml, T. M. Jessell, A role for the roof plate and its resident TGFβ-related proteins in neuronal patterning in the dorsal spinal cord. *Cell* **91**, 127–138 (1997). doi: 10.1016/S0092-8674(01)80015-5; pmid: 9335341

77. M. Lek *et al*., A homeodomain feedback circuit underlies step-function interpretation of a Shh morphogen gradient during ventral neural patterning. *Development* **137**, 4051–4060 (2010). doi: 10.1242/dev.054288; pmid: 21062862
78. A. D. Lander, W. C. Lo, Q. Nie, F. Y. Wan, The measure of success: Constraints, objectives, and tradeoffs in morphogen-mediated patterning. *Cold Spring Harb. Perspect. Biol.* **1**, a002022 (2009). doi: 10.1101/cshperspect.a002022; pmid: 20066078
79. F. Hamaratoglu, A. M. de Lachapelle, G. Pyrowolakis, S. Bergmann, M. Affolter, Dpp signaling activity requires Pentagone to scale with tissue size in the growing *Drosophila* wing imaginal disc. *PLOS Biol.* **9**, e1001182 (2011). doi: 10.1371/journal.pbio.1001182; pmid: 22039350
80. R. Vuilleumier *et al*., Control of Dpp morphogen signalling by a secreted feedback regulator. *Nat. Cell Biol.* **12**, 611–617 (2010). doi: 10.1038/ncb2064; pmid: 20453847
81. L. A. Baena-Lopez, H. Nojima, J. P. Vincent, Integration of morphogen signalling within the growth regulatory network. *Curr. Opin. Cell Biol.* **24**, 166–172 (2012). doi: 10.1016/j.ceb.2011.12.010; pmid: 22257639
82. F. Galton, Vox populi. *Nature* **75**, 450–451 (1907).
83. J. Surowiecki, *The Wisdom of Crowds* (Anchor Books/Random House, New York, 2004).
84. S. A. Holley *et al*., A conserved system for dorsal-ventral patterning in insects and vertebrates involving sog and *chordin*. *Nature* **376**, 249–253 (1995). doi: 10.1038/376249a0; pmid: 7617035
85. J. Schmidt, V. Francois, E. Bier, D. Kimelman, *Drosophila* short gastrulation induces an ectopic axis in *Xenopus*: Evidence for conserved mechanisms of dorsal-ventral patterning. *Development* **121**, 4319–4328 (1995). pmid: 8575332
86. R. W. Padgett, J. M. Wozney, W. M. Gelbart, Human BMP sequences can confer normal dorsal-ventral patterning in the *Drosophila* embryo. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2905–2909 (1993). doi: 10.1073/pnas.90.7.2905; pmid: 8464906
87. Y. Goltsev *et al*., Evolution of the dorsal-ventral patterning network in the mosquito, *Anopheles gambiae*. *Development* **134**, 2415–2424 (2007). doi: 10.1242/dev.02863; pmid: 17522157
88. Y. Akiyama-Oda, H. Oda, Axis specification in the spider embryo: Dpp is required for radial-to-axial symmetry transformation and sog for ventral patterning. *Development* **133**, 2347–2357 (2006). doi: 10.1242/dev.02400; pmid: 16720876
89. M. D. Molina *et al*., Noggin and noggin-like genes control dorsoventral axis regeneration in planarians. *Curr. Biol.* **21**, 300–305 (2011). doi: 10.1016/j.cub.2011.01.016; pmid: 21295481
90. M. A. Gaviño, P. W. Reddien, A Bmp/Admp regulatory circuit controls maintenance and regeneration of dorsal-ventral polarity in planarians. *Curr. Biol.* **21**, 294–299 (2011). doi: 10.1016/j.cub.2011.01.017; pmid: 21295483
91. P. W. Reddien, A. L. Bermange, A. M. Kicza, A. Sánchez Alvarado, BMP signaling regulates the dorsal planarian midline and is needed for asymmetric regeneration. *Development* **134**, 4043–4051 (2007). doi: 10.1242/dev.007138; pmid: 17942485
92. F. Lapraz, L. Besnardeau, T. Lepage, Patterning of the dorsal-ventral axis in echinoderms: Insights into the evolution of the BMP-chordin signaling network. *PLOS Biol.* **7**, e1000248 (2009). doi: 10.1371/journal.pbio.1000248; pmid: 19956794
93. J. K. Yu *et al*., Axial patterning in cephalochordates and the evolution of the organizer. *Nature* **445**, 613–617 (2007). doi: 10.1038/nature05472; pmid: 17237766
94. E. Bier, Evolution of development: Diversified dorsoventral patterning. *Curr. Biol.* **21**, R591–R594 (2011). doi: 10.1016/j.cub.2011.06.037; pmid: 21820625
95. G. Genikhovich *et al*., Axis patterning by BMPs: Cnidarian network reveals evolutionary constraints. *Cell Rep.* **10**, 1646–1654 (2015). doi: 10.1016/j.celrep.2015.02.035
96. C. Niehrs, On growth and form: A Cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes. *Development* **137**, 845–857 (2010). doi: 10.1242/dev.039651; pmid: 20179091
97. J. W. Valentine, *On The Origin of Phyla* (Univ. of Chicago Press, Chicago, ed. 1, 2004).
98. C. J. Lowe *et al*., Dorsoventral patterning in hemichordates: Insights into early chordate evolution. *PLOS Biol.* **4**, e291 (2006). doi: 10.1371/journal.pbio.0040291; pmid: 16933975
99. M. van der Zee, O. Stockhammer, C. von Levetzow, R. Nunes da Fonseca, S. Roth, Sog/Chordin is required for ventral-to-dorsal Dpp/BMP transport and head formation in a short germ insect. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16307–16312 (2006). doi: 10.1073/pnas.0605154103; pmid: 17050690
100. H. L. Ashe, M. Levine, Local inhibition and long-range enhancement of Dpp signal transduction by Sog. *Nature* **398**, 427–431 (1999). doi: 10.1038/18892; pmid: 10201373
101. E. Bier, Developmental biology: A unity of opposites. *Nature* **398**, 375–376 (1999). pmid: 10201364
102. K. Yu *et al*., Cysteine repeat domains and adjacent sequences determine distinct bone morphogenetic protein modulatory activities of the *Drosophila* Sog protein. *Genetics* **166**, 1323–1336 (2004). doi: 10.1534/genetics.166.3.1323; pmid: 15082551
103. E. Decotto, E. L. Ferguson, A positive role for Short gastrulation in modulating BMP signaling during dorsoventral patterning in the *Drosophila* embryo. *Development* **128**, 3831–3841 (2001). pmid: 11585808
104. E. L. Ferguson, K. V. Anderson, Localized enhancement and repression of the activity of the TGF-β family member, *decapentaplegic*, is necessary for dorsal-ventral pattern formation in the *Drosophila* embryo. *Development* **114**, 583–597 (1992). pmid: 1618130
105. E. L. Ferguson, Conservation of dorsal-ventral patterning in arthropods and chordates. *Curr. Opin. Genet. Dev.* **6**, 424–431 (1996). doi: 10.1016/S0959-437X(96)80063-3; pmid: 8791529
106. D. M. Umulis, M. Serpe, M. B. O'Connor, H. G. Othmer, Robust, bistable patterning of the dorsal surface of the *Drosophila* embryo. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 11613–11618 (2006). doi: 10.1073/pnas.0510398103; pmid: 16864795
107. A. M. Rafiqi, C. H. Park, C. W. Kwan, S. Lemke, U. Schmidt-Ott, BMP-dependent serosa and amnion specification in the scuttle fly *Megaselia abdita*. *Development* **139**, 3373–3382 (2012). doi: 10.1242/dev.083873; pmid: 22874914
108. Y. Akiyama-Oda, H. Oda, Early patterning of the spider embryo: A cluster of mesenchymal cells at the cumulus produces Dpp signals received by germ disc epithelial cells. *Development* **130**, 1735–1747 (2003). doi: 10.1242/dev.00390; pmid: 12642480
109. D. Bachiller *et al*., The organizer factors Chordin and Noggin are required for mouse forebrain development. *Nature* **403**, 658–661 (2000). doi: 10.1038/35001072; pmid: 10688202
110. A. Lauri *et al*., Development of the annelid axochord: Insights into notochord evolution. *Science* **345**, 1365–1368 (2014). pmid: 25214631
111. D. H. Kuo, D. A. Weisblat, A new molecular logic for BMP-mediated dorsoventral patterning in the leech *Helobdella*. *Curr. Biol.* **21**, 1282–1288 (2011). doi: 10.1016/j.cub.2011.06.024; pmid: 21782437
112. S. V. Nuzhdin *et al*., Natural genetic variation in transcriptome reflects network structure inferred with major effect mutations: Insulin/TOR and associated phenotypes in *Drosophila melanogaster*. *BMC Genomics* **10**, 124 (2009). doi: 10.1186/1471-2164-10-124; pmid: 19317915
113. I. Dworkin *et al*., The effects of weak genetic perturbations on the transcriptome of the wing imaginal disc and its association with wing shape in *Drosophila melanogaster*. *Genetics* **187**, 1171–1184 (2011). doi: 10.1534/genetics.110.125922; pmid: 21288875
114. E. Bier, *The Coiled Spring: How Life Begins* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, ed. 1, 2000).
115. D. Kosman *et al*., Multiplex detection of RNA expression in *Drosophila* embryos. *Science* **305**, 846 (2004). doi: 10.1126/science.1099247; pmid: 15297669
116. J. C. Illes, E. Winterbottom, H. V. Isaacs, Cloning and expression analysis of the anterior parahox genes, *Gsh1* and *Gsh2* from *Xenopus tropicalis*. *Dev. Dyn.* **238**, 194–203 (2009). doi: 10.1002/dvdy.21816; pmid: 19097192
117. T. B. Kornberg, S. Roy, Cytonemes as specialized signaling filopodia. *Development* **141**, 729–736 (2014). doi: 10.1242/dev.086223; pmid: 24496611
118. E. Bier, Drawing lines in the *Drosophila* wing: Initiation of wing vein development. *Curr. Opin. Genet. Dev.* **10**, 393–398 (2000). doi: 10.1016/S0959-437X(00)00102-7; pmid: 10889058



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