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Control of Tissue Development by Morphogens

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Keywords

morphogen, gene regulatory network, tissue development, diffusion, embryo

Abstract

Intercellular signaling molecules, known as morphogens, act at a long range in developing tissues to provide spatial information and control properties such as cell fate and tissue growth. The production, transport, and removal of morphogens shape their concentration profiles in time and space. Downstream signaling cascades and gene regulatory networks within cells then convert the spatiotemporal morphogen profiles into distinct cellular responses. Current challenges are to understand the diverse molecular and cellular mechanisms underlying morphogen gradient formation, as well as the logic of downstream regulatory circuits involved in morphogen interpretation. This knowledge, combining experimental and theoretical results, is essential to understand emerging properties of morphogen-controlled systems, such as robustness and scaling.

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INTRODUCTION

The formation of multicellular organisms requires the spatially and temporally organized generation of multiple distinct cell types. One way this is achieved is through the action of intercellular signals, known as morphogens. The concept of morphogens as organizers of developing tissues and providers of positional information dates back more than a century and remains one of the most influential ideas in developmental biology (for a summary of this history, see Simsek & Özbudak 2022 and Stapornwongkul & Vincent 2021).

The initial identification of molecules responsible for tissue patterning (Driever & Nüsslein-Volhard 1988, Steward et al. 1988) led to the view that morphogens are molecules that spread from localized sources to form concentration gradients and control cell fate in a concentration-dependent manner. Subsequent advances in our understanding of tissue development suggest a more versatile picture of how morphogen gradients are established and function. For example, morphogen gradients can form from distributed sources, be interpreted in a concentration-independent manner, and control cell behaviors other than cell fate specification. Here, we adopt a broad view of morphogens as molecules that act at a long range to control cell properties and provide spatial information for tissue development.

It is apparent that a relatively small number of signals are used repeatedly as morphogens to coordinate the development of multiple tissues, and much of our current understanding is based on studies of a selection of these examples (**Figure 1**). We use these examples to discuss the mechanisms by which morphogen gradients form and how receiving cells transduce morphogen signals and transform them into distinct and precise cellular responses. We compare examples from different species and molecular systems to identify shared features as well as highlight distinctions between commonly studied systems. In doing this, we emphasize the insight that is gained from the application of new technology and the importance of a dialogue between experimental and theoretical work.

MORPHOGEN GRADIENT FORMATION

The hallmark of a morphogen is that it conveys information about position to a tissue. In contrast to Turing-like reaction-diffusion systems, which are not inherently oriented, morphogens form

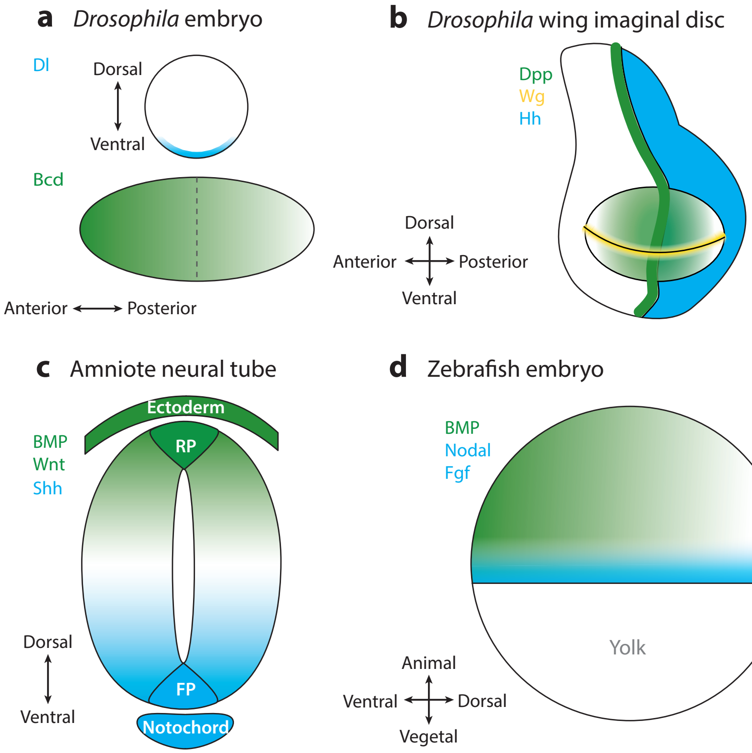
a Drosophila embryo
Dl
Dorsal
Ventral
Bcd
Anterior
Posterior
b Drosophila wing imaginal disc
Dpp
Wg
Hh
Dorsal
Anterior
Posterior
Ventral
c Amniote neural tube
Ectoderm
RP
BMP
Wnt
Shh
Dorsal
Ventral
FP
Notochord
d Zebrafish embryo
BMP
Nodal
Fgf
Yolk
Animal
Ventral
Dorsal
Vegetal

Figure 1

Example model systems and key morphogen gradients. (*a*) *Drosophila* blastoderm patterning is controlled by a Dorsal (Dl, *blue*) morphogen gradient along the dorsal-ventral axis and a Bcd (*green*) anterior-posterior gradient. The upper panel represents a cross section through the embryo (*dashed line* in *lower panel*). The lower panel represents a lateral view. (*b*) Morphogen gradients in the *Drosophila* wing imaginal disc are shown (*top*). Hh (*blue*) is expressed in the posterior compartment and forms a gradient along the anterior-posterior axis. Dpp (*green*) is expressed in response to the Hh gradient in the anterior compartment and forms an anterior-posterior concentration gradient. Wg (*yellow*) is expressed along the dorsal-ventral boundary in third-instar larvae. (*c*) Pattern formation along the dorsal-ventral axis of the vertebrate neural tube depends on opposing morphogen gradients. Ligands from the BMP and Wnt families (*green*) produced in the ectoderm and roof plate form a dorsal to ventral gradient. Shh (*blue*) produced from the notochord and floor plate forms a ventral to dorsal gradient. (*d*) Zebrafish embryo development at stages just prior to and during gastrulation depends on gradients of Nodal and Fgf (*blue*) emanating from the margin of the embryo. An orthogonal ventral to dorsal gradient of BMP activity (*green*) determines patterning along the dorsal-ventral axis. Abbreviations: FP, floor plate; RP, roof plate.

concentration gradients and act along a defined axis within a tissue (Green & Sharpe 2015). The shape and spatiotemporal dynamics of a morphogen concentration profile depend on three factors: production, transport, and removal.

1. Morphogen production can occur from a localized or a distributed source (**Figure 2*a***). Production from a localized source generates a flux of morphogen across the boundary from the source to the target tissue. In mathematical models, this can be captured by the boundary conditions of the morphogen transport equation or by explicitly incorporating the morphogen source (reviewed in Wartlick et al. 2009). In contrast, for a distributed source, an explicit understanding of the processes that govern the formation, and thereby

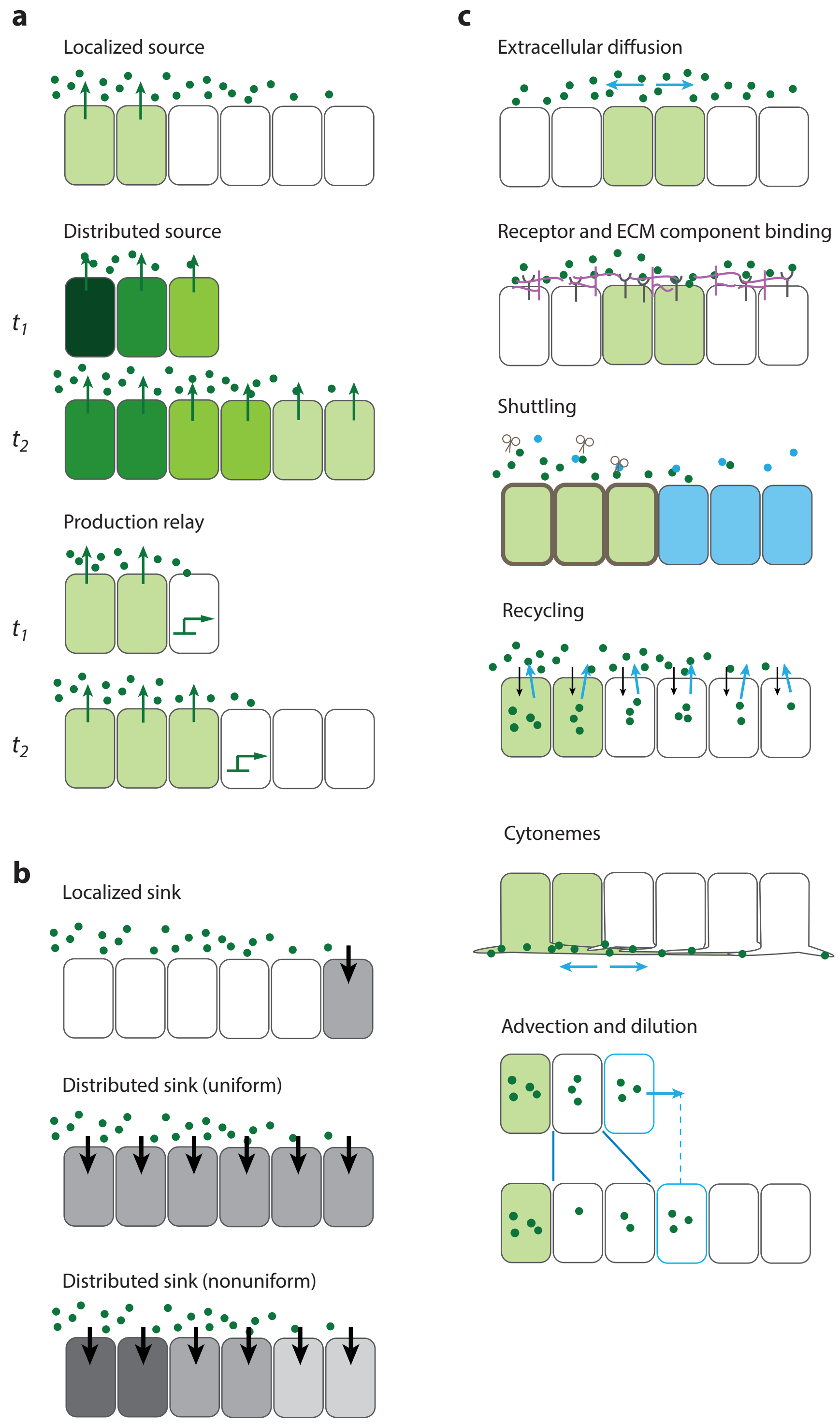
a
Localized source
Distributed source
t_1
t_2
Production relay
t_1
t_2
b
Localized sink
Distributed sink (uniform)
Distributed sink (nonuniform)
c
Extracellular diffusion
Receptor and ECM component binding
Shuttling
Recycling
Cytonemes
Advection and dilution

Figure 2

Cellular mechanisms of morphogen production, transport, and removal in developing tissues. (*a*) Morphogen production can occur in a restricted group of cells (localized source), throughout the tissue (distributed source), or via a relay mechanism that leads to a continuous expansion of the source. t_1 and t_2 are time 1 and 2, respectively. (*b*) Morphogen degradation can be localized or distributed and occur with uniform or variable rates across the tissue. (*c*) Morphogens can spread nondirectionally via extracellular diffusion. Diffusion is often hindered by the binding of morphogens to receptors and components of the ECM, as well as to secreted proteins and chaperones, which may affect morphogen diffusion and activity. Intracellular trafficking mechanisms, such as recycling and cytoneme localization, can influence morphogen gradient formation. Tissue growth leads to directional displacement (advection; *blue arrow*) and dilution of morphogen molecules upon cell division. Abbreviation: ECM, extracellular matrix.

the spatiotemporal characteristics of the source, is needed (Dalessi et al. 2012, Teimouri & Kolomeisky 2015).

2. Morphogen transport can take place via directional or nondirectional mechanisms (**Figure 2*b***). Most known cases of morphogen transport involve nondirectional spreading, typically a randomly oriented diffusion-like motion of molecules through the tissue. Directional spreading occurs when morphogen molecules are actively transported in a specific direction. This can occur, for instance, in a growing tissue, where morphogen molecules are transported away from a fixed point by tissue growth (a process known as advection). Both directional and nondirectional factors may contribute to the overall spreading of morphogen. Regardless of whether spreading is directional or not, the speed with which molecules traverse a certain distance may be constant or vary in space or time. This has consequences for the shape of the morphogen concentration profile.
3. Morphogen can be removed from the tissue via a localized sink or across the tissue (**Figure 2*c***). Removal can occur at a constant or variable rate at different positions across the tissue. Although clearance is often associated with the degradation of morphogen molecules, any mechanism that reduces the amount of morphogen is relevant for gradient formation. This may include internalization or movement into compartments where the morphogen molecules are immobilized or unavailable for further signaling. In addition, dilution of morphogen concentration can occur due to cell division and tissue growth.

Knowledge of these three factors (production, transport, and removal) allows tissue-scale biophysical quantitative descriptions of the changes in morphogen concentration in time and space (Bollenbach et al. 2007, Umulis & Othmer 2015, Wartlick et al. 2009). For morphogens secreted from a localized source, spreading nondirectionally, and being degraded uniformly throughout the tissue, this has become known as the synthesis, diffusion, and degradation (SDD) model (Gregor et al. 2007b, Kicheva et al. 2007, Shvartsman & Baker 2012). It yields steady-state gradients with an exponential shape, characterized by an amplitude (the concentration at the source boundary) and a decay length (the position at which the concentration decays to $1/e$ of the amplitude). In such models, the diffusion coefficient and rates of production and degradation represent the combined behavior of all contributing cellular and molecular processes. A key challenge in studying morphogen gradient formation is understanding how the individual cellular processes give rise to these effective parameters and hence the tissue-scale behavior of the morphogen. Below, we review the current understanding of how morphogen production, spreading, and degradation are controlled.

Regulation of Morphogen Production

Morphogen production most often occurs from a spatially restricted source defined by a developmental prepattern. Morphogen-producing cells can be localized to a dedicated tissue: For example, the vertebrate notochord produces Shh, which patterns adjacent tissues (**Figure 1*c***) (Echelard et al. 1993). Alternatively, morphogen-producing cells can be specified within the tissue being patterned. The continuous specification of morphogen-producing cells and tissue growth can lead to an increase in the size of the source. Examples include the Dpp- and Hh-producing domains in the fly wing disc (Wartlick et al. 2011) and the floor plate of the neural tube (Kicheva et al. 2014). An increasing source size may contribute to an increase in the overall amount of morphogen produced in the tissue; however, the production rate that is relevant to gradient formation in the target tissue also depends on the tissue geometry and the production rate per cell.

A morphogen source can also expand or move via a relay mechanism in which morphogen signaling induces its own expression. In such cases, the range of morphogen distribution depends on how effectively morphogen signaling stimulates morphogen production (Dickmann et al. 2022).

Stronger coupling increases the range of morphogen production and can result in very long-range gradients. Hence, relay mechanisms may be particularly relevant in adult or regenerating tissues, such as salamander limbs and flatworms, which are substantially larger than developing tissues patterned by embryonic morphogen gradients. Nevertheless, relays have been described in developing tissues. In the *Drosophila* eye imaginal disc, Hh induces its own expression in adjacent cells, producing a traveling wave of morphogen expression that sweeps across the tissue to trigger photoreceptor cell differentiation (Míguez et al. 2022). A relay mechanism has also been demonstrated for Nodal in an in vitro model of human germ layer patterning. In this system, comprising colonies of embryonic stem cells grown on micropatterns, Nodal activity spreads by inducing neighboring cells to express Nodal (Liu et al. 2022). The simultaneous induction of the Nodal inhibitor Lefty restricts the range of Nodal signaling.

While in many cases morphogen sources grow over time, the converse has also been observed. For instance, Wg is initially expressed throughout the *Drosophila* wing disc and then gradually constricts to the dorsal-ventral boundary. This generates a temporal gradient of Wg such that cells at the lateral edge of the pouch receive Wg for a shorter period than cells at the dorsal-ventral boundary (Klein & Arias 1998). This suggests an explanation for the surprising result that a membrane-tethered version of Wg can substitute for the endogenous secreted factor (Alexandre et al. 2014). A similar strategy also appears to contribute to the dynamics of BMP signaling in the early zebrafish embryo. Several BMP ligands are initially ubiquitously expressed in the blastula but are progressively repressed from dorsal tissue to become restricted to the ventral side of the embryo (Ramel & Hill 2013).

A morphogen source can also appear to move across a tissue through an inheritance mechanism in which cell division carries the morphogen ligand or messenger RNA encoding the morphogen away from its site of deposition, progressively diluting it at every division. This has been observed for Wg in the *Drosophila* embryo (Pfeiffer et al. 2000) and for FGF in vertebrate presomitic mesoderm (Dubrulle & Pourquié 2004). A similar principle also operates in plant roots. As the root grows, cells containing auxin are displaced away from the tip, and the levels of auxin decline to produce a gradient of activity (Mähönen et al. 2014). High levels of auxin in the root tip are maintained by a reflux loop in which PIN proteins transport auxin back towards the tip (Blilou et al. 2005, Galinha et al. 2007, Grieneisen et al. 2007).

Cellular Mechanisms of Morphogen Transport

Nondirectional morphogen transport can be described as a diffusion-like process, and it can be modeled using a diffusion equation in which the diffusion coefficient (D) represents how fast morphogen molecules move in the tissue (Wartlick et al. 2009). At the nanometer/millisecond scale, the diffusion coefficient of molecules in liquids is well described by the Stokes-Einstein equation ($D = \frac{k_B T}{6\pi \eta r}$), where k_B is the Boltzmann constant. Thus, D depends on the temperature T, the size of the diffusing molecule r, and the viscosity η of the environment. However, at the scale of the tissue (micrometers to millimeters), morphogen movement is affected by multiple factors. These include the tortuosity of extracellular space resulting from cell packing, the binding and unbinding of morphogen to cell surface receptors and extracellular matrix (ECM) components, and the trafficking of morphogens in to and out of cells (**Figure 2*b***). The result is an effective diffusion coefficient, reflecting all events that affect morphogen spreading (for a summary, see Müller et al. 2013).

Different methods for measuring morphogen spreading, such as fluorescence correlation spectroscopy (FCS) and fluorescence recovery after photobleaching (FRAP), provide estimates at different temporal and spatial scales (reviewed in Kicheva et al. 2012). The short length scales (femtoliter) and timescales (seconds) of FCS mean that it captures the local diffusivity of individual molecules. By contrast, FRAP quantifies the movement of a population of morphogen molecules across larger distances (micrometers) over longer timescales (minutes to hours). Because of their different length scales and timescales, FCS and FRAP often yield different estimates of the diffusion coefficient and degradation rate. For instance, when measured with FRAP, the diffusion coefficient of Bicoid (Bcd) was estimated as $\sim 0.3\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$. This is an order of magnitude slower than a similarly sized molecule that diffuses freely in water (Gregor et al. 2007b). In contrast, measurements using FCS (Abu-Arish et al. 2010) indicated that Bcd diffused at $5\text{–}10\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$.

The interaction of morphogens with the complex environment of tissues creates pools of molecules with different mobilities. Molecules that are bound to cell surface receptors, for example, are temporarily immobilized. For FGF8 in the early zebrafish embryo, FCS indicated that the majority of FGF8a molecules (92%) displayed fast movement with $D = 56\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$, while a second population moved an order of magnitude slower, $D = 4\ \mu\mathrm{m}^2\ \mathrm{s}^{-1}$ (Harish et al. 2022). Removing extracellular glycoproteins from the tissue decreased the amount of morphogens in the slow-moving fraction and broadened the range of FGF8a activity. This supports the idea that FGF8a spreads via diffusion through the extracellular space and is hindered by interactions with glycoproteins. Fast- and slow-moving fractions of Bcd have also been observed in the cytoplasm and nucleus of the *Drosophila* blastoderm (Athilingam et al. 2022). In the nucleus, the slow pool of Bcd emerged due to DNA binding. In the cytoplasm, it is unclear what slows Bcd movement—one possibility could be interactions with cytoskeletal components. Below we discuss typical examples of morphogen binding proteins that affect morphogen movement.

Morphogen binding proteins. Morphogens bind to receptors that slow morphogen movement as they spread through tissue (for a review, see Stapornwongkul & Vincent 2021). For example, in the early zebrafish embryo, monitoring individual morphogen molecules in real time revealed mobile and immobile fractions of TGFβ superfamily members Nodal and Lefty (Kuhn et al. 2022). The diffusion coefficient of the mobile fractions was similar for Nodal and Lefty, consistent with their similar size. However, Nodal ligands stayed bound to cell surfaces for tens of seconds, whereas Lefty ligands had fewer and shorter binding events. The overexpression of the Nodal cell surface receptor One Eyed Pinhead (OEP) increased the proportion of immobile Nodal molecules, suggesting that OEP was partly responsible for hindering Nodal movement. This was further confirmed by experiments that indicated that the range of endogenous Nodal signaling is set by OEP (Lord et al. 2021). This suggests that the differential binding of Nodal and Lefty proteins to extracellular binding partners alters their effective diffusivity and explains the shorter range of Nodal activity compared to that of Lefty (Müller et al. 2012).

The expression of receptors can be controlled by morphogen signaling, introducing feedback. This can profoundly affect the shape of morphogen gradients over time (Eldar et al. 2003). For example, Hh signaling induces the expression of Hh receptors such as Ptch1. This results in increased sequestration and degradation of the ligand. This mechanism dampens Hh signaling over time and shortens its range (Jeong & McMahon 2005), but it also increases the robustness to fluctuations in morphogen production (Eldar et al. 2003). A similar effect was observed in a synthetic system in which a gradient of Shh was reconstituted in NIH3T3 cells (Li et al. 2018). In contrast to Hh, Dpp and Wg in the *Drosophila* wing disc downregulate their receptor expression, and this has been proposed to minimize sequestration near the morphogen source to allow increased diffusion (Cadigan et al. 1998, Lander et al. 2009, Lecuit & Cohen 1998).

Besides high-affinity receptors, morphogens also bind with lower affinities to ECM components. Transmembrane glycoproteins, particularly heparan sulfate proteoglycans (HSPGs), are central to morphogen transport in many tissues. They influence the spread of members of the Wnt, BMP, Hh, and FGF families (Häcker et al. 1997, Haerry et al. 1997, The et al. 1999, Yayon et al. 1991). Some HSPGs (e.g., perlecan and collagen XVIII) are secreted into the extracellular space, whereas others are associated with the cell membrane either via a transmembrane domain (syndecans) or via a glycosylphosphatidylinositol (GPI) anchor (glypicans) (Nakato & Li 2016). The negatively charged glycosaminoglycan chains on HSPGs provide many low-affinity binding sites (Cardin & Weintraub 1989), which mediate transient interactions that slow the effective morphogen diffusion.

How the combination of high-affinity signaling receptors and low-affinity nonsignaling morphogen binding proteins contributes to shaping the gradients of morphogen signaling activity is a key question. Beside altering the kinetics of movement, low-affinity interactions with HSPGs can serve to internalize morphogens into the endocytic pathways (Romanova-Michaelides et al. 2022). HSPGs can also act as coreceptors to increase intracellular signaling either by forming a stable complex between ligand, HSPG, and receptor, as is the case for FGF (Nugent & Edelman 1992), or by increasing the local concentration of a ligand at the cell surface (Akiyama et al. 2008). A recent study in which GFP was repurposed as a synthetic morphogen in *Drosophila* wing discs showed that the binding affinity of surface proteins for the morphogen determined both the total amount of morphogen accumulating in the tissue and the range of morphogen spreading (Stapornwongkul et al. 2020). The higher the affinity of ligand binders, the less was lost from the tissue but the shorter the range of the gradient. The relative importance of retaining versus slowing morphogen molecules could explain the opposing roles ascribed to HSPGs in different tissues. For example, HSPGs have been implicated in restricting the signaling range of BMPs in vertebrate tissues (Hu et al. 2004, Ohkawara et al. 2002), but in *Drosophila* imaginal discs, HSPGs appear to extend the signaling range of the BMP ortholog Dpp (Bornemann et al. 2004, Fujise et al. 2003).

Morphogens also bind to secreted molecules. For instance, BMP binds to Chordin, which blocks its interaction with the BMP receptor. The Chordin–BMP complex is thought to be highly diffusible and is degraded by the extracellular protease Tolloid, allowing BMP to signal (Umulis et al. 2009). This led to the idea that the movement of Chordin–BMP complexes results in the shuttling of complexes to regions of Chordin degradation where BMP becomes immobile and signaling can occur (Eldar et al. 2002). The shuttling of Dpp has been proposed to play a crucial role in concentrating DPP into a peak at the dorsal midline of the *Drosophila* embryo (Shimmi et al. 2005). Similar mechanisms have been proposed for Chordin–BMP in frog (Ben-Zvi et al. 2008) and zebrafish embryos (Pomreinke et al. 2017, Zinski et al. 2017). In zebrafish embryos, quantitative imaging and mathematical modeling indicated that BMP and Chordin diffuse rapidly to form extracellular protein gradients, as anticipated (Pomreinke et al. 2017, Zinski et al. 2017). However, Chordin did not appear to change the diffusivity or spread of BMP. This ruled out a major contribution for shuttling. Instead, the BMP gradient appears to form by a source-sink mechanism in which BMP spreads by extracellular diffusion and is antagonized by Chordin, emanating from the opposite side of the embryo, which functions as a sink. Consistent with this, a membrane-tethered version of Chordin, unable to diffuse, is sufficient to substitute for wild-type Chordin (Tuazon et al. 2020). Hence, despite the apparent equivalence of molecular components and organization, similar systems can function in different ways in different tissues.

Intracellular trafficking. In addition to binding and unbinding to extracellular proteins, the internalization of cell surface–bound morphogen influences morphogen movement. Morphogen endocytosis is followed by intracellular trafficking, which may include degradation and recycling of the morphogen. In the *Drosophila* wing disc, recycling of Dpp has been observed by photoconverting the intracellular fraction of morphogen and tracking its spread away from the site of photoconversion (Romanova-Michaelides et al. 2022). In this system, the relative contribution of recycling and extracellular diffusion to Dpp transport is determined by the expression level of the extracellular molecule Pentagone, which modulates the binding of Dpp to surface receptors and its extracellular diffusivity. Quantitative analysis of the distinct cellular processes showed that the relative contribution of recycling increases over the course of development as the wing disc grows (Romanova-Michaelides et al. 2022). This allows the Dpp gradient to expand over time and scale with tissue size. Besides its implications for scaling, morphogen recycling can create nonlinear dynamics in morphogen transport in certain regimes and could increase the robustness of gradient formation (Bollenbach et al. 2007).

Cytonemes. Another mechanism proposed to affect morphogen spread involves specialized actin-based filopodia termed cytonemes (Brunt et al. 2021, Du et al. 2018, Mattes et al. 2018, Patel et al. 2022, Ramírez-Weber & Kornberg 1999, Sanders et al. 2013). Secreted morphogens and components of their signaling pathways have been observed in cytonemes (Bischoff et al. 2013, Mattes et al. 2018, Sanders et al. 2013, Stanganello et al. 2015), and cytoneme-mediated interaction between source and target cells has been suggested to allow the transfer of morphogens through specific contacts termed morphogenetic synapses (Kornberg 2017). For example, in zebrafish embryos, Wnt8a has been observed on the tips of actin-based filopodia activating signaling in adjacent cells (Stanganello et al. 2015). Cytoneme formation and morphogen signaling range have been shown to decrease or increase in response to blocking or promoting actin cytoskeleton activity, respectively (Bischoff et al. 2013, Roy et al. 2014, Stanganello et al. 2015). In this view, cytonemes, similar to HSPGs, extend morphogen activity range. Moreover, live imaging of cytonemes in *Drosophila* has revealed periods of extension and contraction. This has been suggested to generate a distribution of cytoneme lengths that could underlie formation of an Hh concentration gradient (Aguirre-Tamaral & Guerrero 2021, Bischoff et al. 2013). However, quantitative data that address whether cytonemes can transport sufficient amounts of morphogen at sufficient distances to generate the observed gradients are needed. More specific means to interfere with cytoneme formation would also test the significance of cytonemes in morphogen gradient formation. Broadly blocking cytoskeletal activity is likely to have pleiotropic effects. Targeting reagents to block cytoneme formation just in the appropriate region of a cell, perhaps using optogenetic tools, would provide better functional assays.

Lipid-modified morphogens. Lipid-modified morphogens, such as Hh and Wnt family members, require specific mechanisms that allow them to move through the aqueous extracellular space. Hh proteins have been suggested to assemble into higher-order multimeric micelle structures with a lipophilic core secluded from the hydrophilic surface (Chen et al. 2004, Zeng et al. 2001). Alternatively, lipidated morphogens might insert their lipid moiety into the outer membranes of exosomes or lipoprotein particles (Gross et al. 2012, Korkut et al. 2009, Liégeois et al. 2006, Matusek et al. 2014, Panáková et al. 2005, Vyas et al. 2014), which then allow these ligands to be transported through tissues. It has, however, been difficult to establish the contribution of these mechanisms to morphogen spread. The size of the proposed higher-order structures would be expected to substantially reduce their diffusion. Visualizing and testing the role of these complexes have been challenging. In the wing imaginal disc, imaging alleles of Wg tagged with different markers argued against the involvement of multiprotein complexes (McGough et al. 2020). Further experiments, such as fluorescence cross-correlation spectroscopy to determine if two molecules labeled with different fluorophores move together, are needed to determine whether morphogen molecules move as complexes or as individual molecules.

Specific chaperone proteins that facilitate morphogen spreading by shielding the lipid moieties have also been identified. In *Drosophila*, Wnt family members are chaperoned by Secreted Wingless-Interacting Molecule (SWIM) and Carrier of Wingless (Cow), while Wnt inhibitory factor (WIF) and Afamin have been identified in vertebrates (Chang & Sun 2014, Hsieh et al. 1999, Leyns et al. 1997, Mihara et al. 2016, Mulligan et al. 2012). HSPGs also appear to contribute to lipid shielding. The GPI anchor of HSPGs, such as Dlp, retains Wnt family members within the tissue but allows diffusion along the cell membrane. This assists Wnt spreading and interaction with signaling receptors (McGough et al. 2020). For Hh family members, Shifted has been identified in *Drosophila* (Gorfinkiel et al. 2005). In vertebrates, the spread of Shh requires two solubilizing factors: the membrane protein Dispatched1 (Disp1) (Burke et al. 1999, Caspary et al. 2002, Kawakami et al. 2002, Ma et al. 2002), which promotes the release of lipidated Shh from producing cells, and Scube2 (Hollway et al. 2006, Kawakami et al. 2005, Woods & Talbot 2005), which chaperones Shh after release by shielding the lipid adducts (Tukachinsky et al. 2012). Shh bound to Scube2 is unable to signal. A mechanism involving the cell surface proteins Cdon, Boc, and Gas1 is responsible for dissociating Shh from Scube2 and transferring the ligand to Ptch1, which initiates signaling (Wierbowski et al. 2020).

Effects of tissue growth on gradient formation. Tissue growth or morphogenesis can affect morphogen transport by displacing cells, and consequently the morphogen molecules they contain, with respect to the morphogen source. This is known as advection. Furthermore, cell proliferation leads to a dilution of the morphogen molecules. Advection and dilution occur alongside other mechanisms that shape morphogen gradient profiles, and therefore, biophysical descriptions of morphogen gradient formation in growing tissues take these effects explicitly into account (Averbukh et al. 2014, Bittig et al. 2009, Fried & Iber 2014, Fulton et al. 2022). The contribution of advection and diffusion to gradient formation depends on the rates of growth and kinetic parameters of morphogen transport and may change over developmental time. For instance, a model capturing cell movements during zebrafish epiboly suggests that advection is likely to have a significant contribution to BMP gradient formation after 50% epiboly (Li et al. 2022). Similarly, the motility and rearrangement of cells in the presomitic mesoderm have been suggested to influence the dynamics of FGF and Wnt signaling in individual cells and thereby the pattern of differentiation (Fulton et al. 2021, 2022). Tissue morphogenesis may result in specific tissue architecture that influences morphogen spread, for instance, by confining morphogen molecules within a given space. An example of this has been reported in the developing vertebrate intestine, where Shh is uniformly expressed in the endoderm but the curved villus tip is proposed to increase the local Shh concentration close to the tip (Shyer et al. 2015).

Regulation of Morphogen Degradation

The degradation rate of morphogens has a critical influence on the timescale of morphogen gradient formation, as well as on the shape of the gradient (Berezhkovskii et al. 2010, Wartlick et al. 2009). In the SDD model, a spatially uniform degradation rate yields a steady-state exponential gradient (Gregor et al. 2007b, Kicheva et al. 2007). Yet, studies in several systems reveal that the degradation rate is often regulated and can vary in both space and time. For instance, the degradation rate of Bcd in the *Drosophila* embryo has been shown to be high (∼25 min) at a specific point of blastoderm development [nuclear cycle (NC) 14], and this is essential for the gradient shape (Durrieu et al. 2018, Little et al. 2011). However, the shape of the Bcd gradient changes dramatically over time: From NC 10 to 13, Bcd levels increase early on; they then decline at NC 13–14 (Bergmann et al. 2007, Gregor et al. 2007b, Little et al. 2011). A tandem fluorescent timer variant of Bcd, in which the difference in the maturation rate between two fluorophores fused to Bcd acts as a protein age sensor, revealed that the production rate decreased and degradation rates increased after NC 13, which could explain the observed dynamics in Bcd levels (Durrieu et al. 2018). This is in line with previous results in which the Bcd degradation rate was shown to substantially increase from NC 14 onwards (Drocco et al. 2011). A consequence is that pattern formation in the blastoderm occurs when the Bcd gradient is not in steady state (Bergmann et al. 2007). Such a mechanism can reduce patterning errors caused by fluctuations in the rate of morphogen production.

Temporal changes in morphogen degradation rates have also been observed for Dpp in the fly wing disc (Romanova-Michaelides et al. 2022, Wartlick et al. 2011). The changes in the effective Dpp degradation over time account for the observed temporal changes in the gradient decay length and amplitude. This allows the gradient to scale to the growing area of the tissue (Wartlick et al. 2011).

Feedback through the morphogen regulation of receptor expression can result in position-dependent internalization and degradation rates. This type of mechanism (self-enhanced ligand degradation) is predicted to result in the formation of a gradient that follows a power law rather than an exponential shape (Bollenbach et al. 2005, Eldar et al. 2003, Li et al. 2018), and this has useful properties that would be expected to increase the robustness of the morphogen gradient shape to fluctuations in morphogen production. Testing these predictions experimentally remains challenging, in part because high-resolution imaging with low levels of experimental variability is necessary to confidently distinguish between a power-law and exponential distribution of a morphogen ligand.

SCALING MORPHOGEN GRADIENTS

Patterning mechanisms need to be sufficiently robust to differences in tissue size between individuals. One possibility for how pattern scaling could be achieved is by scaling the morphogen gradient itself (**Figure 3**). Implicit in this idea is the assumption that target gene expression boundaries are positioned at constant thresholds of morphogen concentration. Thus, scaling would require the positions of the concentration thresholds to scale with the tissue size. For an exponential gradient, scaling the decay length to tissue size while maintaining a constant amplitude would result in perfect scaling of the concentration thresholds (**Figure 3*a***). In several cases where scaling has been studied, however, it appears that the amplitude of the morphogen gradient changes. This would result in deviations from perfect scaling that vary in magnitude depending on the position within the tissue (**Figure 3*b***) (Umulis & Othmer 2013). Nevertheless, mechanisms in which the amplitude changes may still produce nearly perfect scaling throughout much of the tissue. For instance, the amplitude of Bcd in *Drosophila* eggs with different volumes scales with egg size, resulting in scaling of the Bcd gradient throughout broad regions of the embryo (Cheung et al. 2011).

In contrast to intraspecies scaling, scaling of the Bcd gradient between different dipteran species has been attributed to changes in the decay length, rather than amplitude (Gregor et al. 2005). Because the decay length of an exponential gradient depends on the effective diffusion coefficient and degradation rate, scaling implies that these parameters change in a way that is dependent on the tissue size (for a review, see Čapek & Müller 2019). A way of achieving this is via an accessory molecule that modulates morphogen diffusion or degradation and is itself highly diffusive, so that its concentration rapidly adjusts to tissue size (Rasolonjanahary & Vasiev 2016). A potential weakness of such a mechanism is that it is sensitive to fluctuations in the production of the accessory molecule, which would decrease the accuracy of scaling.

Coupling morphogen signaling to the regulation of an accessory molecule via a feedback loop provides a way to adjust morphogen spread to tissue size dynamically. The expansion-repression

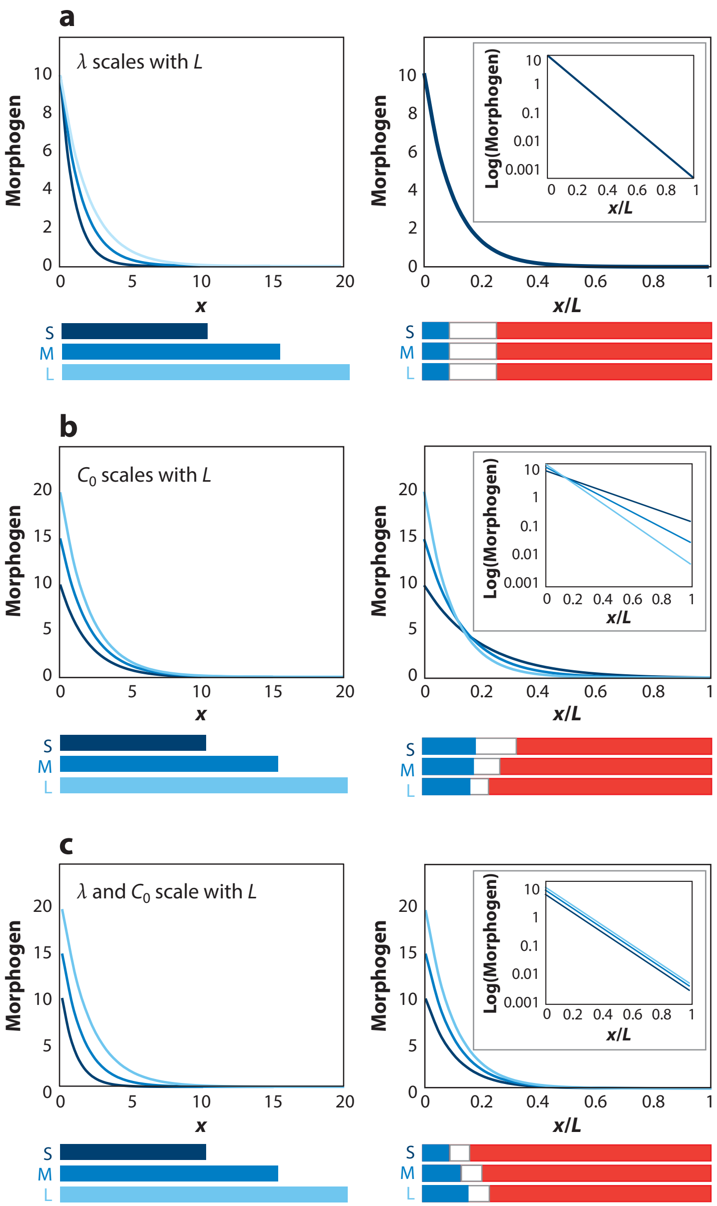

Figure 3

Morphogen gradient scaling and its relationship to pattern scaling. An exponential morphogen gradient in tissues with three different sizes is shown. Left panels show morphogen concentration as a function of the absolute distance x from the source boundary; right panels show x relative to the tissue size L (*insets* show the morphogen concentration on a log scale). The color bars on the right show three gene expression domains with boundaries positioned at constant morphogen concentration thresholds. (*a*) The decay length λ is proportional to tissue size, while the amplitude C_0 stays constant (*left*). In this case, the pattern scales perfectly with tissue size. (*b*) C_0 is proportional to L, and λ is constant. The blue domain boundary occurs always at a similar relative position, while the red domain boundary position varies significantly with tissue size. (*c*) Both λ and C_0 scale with L. In this case, boundary positions vary with tissue size across the entire tissue. Abbreviations: L, large; M, medium; S, small.

mechanism (Ben-Zvi & Barkai 2010) exemplifies this. In this mechanism, a morphogen represses the expression of a secreted expander molecule. The expander in turn facilitates the spread of the morphogen by, for example, reducing its trapping or degradation. This sets up a feedback loop in which the expander promotes morphogen spread. The spread of the morphogen then shuts off expander production, leading to a decrease in morphogen spread. For Dpp in the *Drosophila* wing disc, Pentagone has been shown to support scaling of Dpp signaling in a manner consistent with an expander mechanism (Ben-Zvi et al. 2011, Hamaratoglu et al. 2011, Vuilleumier et al. 2010). Molecularly, Pentagone has been proposed to function by modifying the binding of Dpp to the ECM and cell surface receptors to increase the amount of Dpp being recycled and thus extend the range of Dpp (Romanova-Michaelides et al. 2022). However, other mechanisms have also been proposed to contribute to Dpp scaling in the wing disc, including Dpp-mediated downregulation of its receptors, which extends the gradient range (Zhu et al. 2020), as well as the contribution of cell death to correcting the mismatch between the gradient range and tissue size (Merino et al. 2022). Expansion-repression mechanisms have also been proposed to scale morphogen gradients in vertebrate systems, for instance, the BMP gradient in the pectoral fin (Mateus et al. 2020) as well as the Shh gradient in the ventral neural tube (Collins et al. 2018). Scube2 promotes Shh spread by shielding its lipid moieties, but Shh signaling represses Scube2 expression, thereby establishing an expansion-repression feedback loop.

An equivalent contraction-induction mechanism, in which a contractor molecule is activated by the morphogen and decreases morphogen spread, also provides a way to scale a morphogen gradient. WntD, a secreted protein induced by Toll-dependent signaling during dorsoventral patterning of the *Drosophila* embryo, appears to act as a contractor as it binds to and inhibits the Toll receptor (Rahimi et al. 2020). A related mechanism has been proposed to scale Nodal-dependent endoderm and mesoderm induction in zebrafish (Almuedo-Castillo et al. 2018). The Nodal signaling gradient is controlled by the inhibitor Lefty, which is itself induced by Nodal signaling. Because of the high diffusivity of Lefty, its concentration is lower in larger embryos, which allows the range of Nodal activity to increase. Although this appears to correspond to the contraction-induction mechanism, theoretical and experimental decoupling of the induction of Lefty by Nodal indicated that direct regulation of Lefty by Nodal was not necessary for scaling, suggesting that the system represents a simpler example of control by an accessory molecule (Almuedo-Castillo et al. 2018).

To fully understand the relevance and consequences of morphogen gradient scaling, the mechanism of morphogen interpretation also needs to be considered. For instance, if the temporal dynamics of morphogen signaling are important, then a constant gradient amplitude may not be necessary (and might be inconsistent) with scaling. In the case of Dpp, temporal changes in signaling have been proposed to regulate the rate of proliferation in the *Drosophila* wing disc (Wartlick et al. 2011). In this scenario, the scaling of both the amplitude and decay length of the gradient is important for a spatially uniform proliferation rate. This requires feedback between gradient formation and tissue growth—the gradient amplitude and decay length change with tissue size and at the same time drive tissue growth. The scaling behavior introduced by such feedback depends on the coupling strength between the change in morphogen levels and the division rate (Aguilar-Hidalgo et al. 2018, Averbukh et al. 2014).

The mechanism of morphogen interpretation may also be sensitive to tissue size. For instance, the expression levels of gap genes at the mid-embryo appear to be size dependent (Wu et al. 2015). This emphasizes the importance of considering the interpretation mechanism in understanding how patterns scale between individuals. Given the complexity of mechanisms that are being uncovered, this is an important future research direction. The mechanisms that are emerging begin to explain the function of some of the molecules implicated in the control of morphogen spread and suggest why complexities in the transport mechanisms have evolved. Studies of morphogen scaling thus increasingly emphasize the importance of quantitative data and rigorous theoretical analysis.

MORPHOGEN RECEPTION AND TRANSDUCTION

In a few cases, such as Bcd in *Drosophila* (Driever & Nüsslein-Volhard 1988) and retinoic acid in vertebrate tissues (Bernheim & Meilhac 2020), the morphogen is intracellular and directly regulates gene expression. However, in most cases, morphogen ligands initiate an intracellular signaling cascade by binding to cell surface receptors, which culminates in the regulation of transcription effectors mediating gene expression. A frequent assumption is that morphogen concentration is proportional to the level of intracellular signaling activity. This appears to be the case for some morphogens. A threefold difference in the number of activated Activin receptors results in a threefold difference in the amount of the transcription effector Smad2 in the nucleus in *Xenopus* blastula cells (Shimizu & Gurdon 1999). Likewise, in the wing disc, a gradient of Dpp is converted into a similarly shaped gradient of phosphorylated Mad effector in the nuclei of responding cells (Bollenbach et al. 2008). However, such linear or monotonic relationships are not always observed. A striking example can be seen in vertebrate somitogenesis, where an Fgf gradient generates an oscillatory gradient of ppErk by interacting with an oscillator comprising the Her1–Her7 transcription factors during the periodic formation of segments (Simsek et al. 2023). This raises questions about how the mechanism of signal transduction contributes to the interpretation of morphogen signaling.

In some cases, signal transduction mechanisms can introduce temporal delays. The transcriptional effectors of the Wnt and Hh pathways are irreversibly proteolyzed in the absence of a signal (Barolo & Posakony 2002). Pathway activation depends on the de novo synthesis of new full-length transcriptional effectors and the degradation of previously formed repressors. This results in a delay between changes in ligand levels and downstream transcriptional output. Similarly, the retention of ligand-receptor complexes in endosomes can sustain signaling for extended periods of time (Bökel et al. 2006, Jullien & Gurdon 2005). A consequence of these mechanisms is that transient fluctuations in external ligand concentration are buffered and averaged out.

Signal transduction pathways can also introduce nonlinear signal amplification. For example, the cascade of MAP kinases activated by several tyrosine kinase receptors, including EGF, can amplify a signal, creating a threshold-like response (Huang & Ferrell 1996). This results in ultrasensitivity in which a small change in the input creates an all-or-none change in the output. This has been implicated in converting a graded input from the EGF ligand Sptiz in the *Drosophila* epidermis into a sharp on/off output in the downstream transcriptional effector Yan (Melen et al. 2005).

Conversely, in many signaling pathways, inhibitors of signaling are induced by pathway activation (e.g., Ptch1 for Hh signaling, Axin2 and Notum for Wnt signaling, Sprouty for FGF signaling, and Smad6 and -7 for TGFβ signaling). This introduces negative feedback and leads to pathway adaptation in which the intracellular signaling in cells exposed to constant amounts of morphogen gradually decreases. For instance, Shh signaling initiates negative feedback by inducing the expression of Ptch1 and Hhip1, which sequester and inhibit Shh activity (Briscoe et al. 2001, Jeong & McMahon 2005), and by downregulating Shh coreceptors Gas1, Boc, and Cdon (Allen et al. 2007, Tenzen et al. 2006). In the neural tube, cells are initially sensitive to Shh, which allows low Shh concentrations to activate gene expression, including the negative feedback components. As negative feedback increases, higher levels of Shh ligand are necessary to sustain intracellular signaling. Consequently, cells close to the source of Shh, which are exposed to higher ligand concentrations, signal for longer than those further away that are exposed to lower ligand concentrations. TGFβ signaling, in an in vitro model of human germ layer specification, also appears to undergo adaptation (Sorre et al. 2014, Warmflash et al. 2012).

Signal adaptation provides a mechanism to convert morphogen concentration into a duration of signaling. For both Shh and TGFβ signaling, signal duration is important for cell fate decisions. For Shh signaling, ex vivo assays indicated a role for temporal integration with the cell types associated with higher levels of Shh morphogens requiring longer durations of signaling (Dessaud et al. 2007, 2010). In zebrafish embryos, the timed inhibition of Nodal signaling indicated that cell fate specification also depended on the duration of exposure to Nodal ligands (Hagos & Dougan 2007). Moreover, the use of photoactivatable Nodal receptors revealed that the duration of Nodal signaling in embryos determined the cell fate outcomes with longer durations of signaling favoring the induction of prechordal plate mesoderm over endodermal tissue (Sako et al. 2016).

The importance of signaling duration is a widespread feature of morphogen interpretation. Assays using an optogenetically controlled Bcd protein revealed that cells exposed to the highest Bcd concentration require Bcd for the longest duration (Huang et al. 2017). The duration of BMP signaling has also been implicated in controlling whether differentiating human embryonic stem cells remain pluripotent or adopt mesodermal or extraembryonic fates (Camacho-Aguilar et al. 2022). Temporal integration is not observed in all situations, however. By analyzing the effect of changing the dynamics of signaling in mutant zebrafish embryos, the duration of BMP signaling and the slope of the gradient were ruled out as determining the differential response of target genes (Greenfeld et al. 2021). Instead, target genes responded to distinct levels of the BMP transcriptional effector to establish the spatial pattern of gene expression.

Signal transduction and interpretation may also provide a mechanism to sense the fold change in ligand concentration over time (Adler & Alon 2018). In the *Drosophila* wing disc, the amplitude and decay length of the Dpp gradient increase over time and lead to an increase in signaling. Cell division occurs when Dpp signaling levels in cells have increased by 50%. This suggests that cells use the temporal fold change in morphogen signaling levels to control tissue growth (Wartlick et al. 2011). Sensing of fold change in ligand concentrations has also been reported for Wnt (Goentoro & Kirschner 2009), EGF (Cohen-Saidon et al. 2009), and Nodal (Liu et al. 2022). In the case of Nodal, only rapid increases in Nodal levels efficiently induced Bra expression and mesoderm differentiation in an in vitro model of human gastrulation, whereas slow increases in Nodal were inefficient in eliciting a response (Sorre et al. 2014). In vivo, Nodal appears to increase rapidly and manipulation of the level of Nodal signaling suggested concentration effects (Gritsman et al. 2000), but whether the rate of change of Nodal is spatially uniform in vivo and whether this could be used directly to define the boundaries of gene expression domains are unclear.

Taken together, these studies are refining our view of how morphogens impart positional information and control gene expression. Although there are examples where the concentration of extracellular morphogens is transduced downstream in a relatively linear fashion, this is not always the case. Signaling pathways often introduce nonlinearities in the dynamics of the response. In some cases, the temporal features introduced by the signaling mechanism appear to be exploited by responding cells. Moreover, the mechanism by which a specific morphogen is interpreted can differ between tissues and species.

MORPHOGEN-DEPENDENT GENE REGULATION

How do developing tissues convert morphogen signaling into spatially discrete domains of gene expression? Addressing this question requires an understanding of the mechanisms by which target gene expression is regulated. Much of this regulation occurs at the level of the cis regulatory elements (CREs), also known as enhancers. Several aspects of this regulation have been investigated: the properties of binding sites for morphogen effectors, the combinatorial activity of morphogen effectors and other factors bound to the CREs, and cross-interactions between morphogen target genes.

A straightforward mechanism that could result in distinct target gene expression domains is for CREs to harbor different numbers or affinities of binding sites for the morphogen transcriptional effector. In this way, targets with fewer or lower affinity sites would require higher levels of morphogen signaling, and consequently, their expression would be restricted to regions close to the morphogen source. However, although CRE affinity can influence sensitivity to morphogen (Driever et al. 1989, Jiang & Levine 1993, Wharton et al. 2004), it does not appear to be the case in general, as multiple studies have failed to find a correlation between the strength of morphogen effector binding and activity (Ochoa-Espinosa et al. 2005, Oosterveen et al. 2012, Peterson et al. 2012). Moreover, several morphogen effectors (e.g., in the Wnt and Hh pathways) are bifunctional, acting as repressors in the absence of a signal and as activators in the presence of a signal (Barolo & Posakony 2002). Since both isoforms bind the same CREs, the affinity or number of binding sites affects both activator and repressor binding equally.

For Bcd, more than 60 CREs have been identified (Chen et al. 2012). These do not show a correlation between Bcd binding site number, or affinity, and their responsiveness. Instead, the binding of other transcription factors, including ubiquitously expressed factors, plays an important role in establishing the differential response of different CREs. For instance, the transcription factor Zelda is broadly distributed in the blastoderm. It binds to the CREs of many Bcd targets where it acts as a pioneer factor to potentiate the ability of Bcd to activate gene expression (Foo et al. 2014, Harrison et al. 2011). Moreover, Zelda is found in so-called nuclear hubs together with Bcd (Mir et al. 2018), and it has been suggested to contribute to reducing the time required for Bcd to activate target genes (Fernandes et al. 2022).

Zelda also interacts with targets of Dorsal, the morphogen effector that patterns the dorsal ventral axis of the blastoderm and is proposed to play a similar role (Li & Eisen 2018). In the neural tube, members of the SoxB1 family of transcription factors (Sox1–3) appear to play a similar role to Zelda. SoxB1 factors are expressed in all neural progenitors, bind to Shh-responsive CREs, and appear to facilitate the Shh responsiveness of these CREs (Bergsland et al. 2011, Cohen et al. 2014, Oosterveen et al. 2012, Peterson et al. 2012). These examples illustrate how the responsiveness of a morphogen target gene can be modified independent from the simple binding affinity of CREs for the morphogen effector itself (Kanodia et al. 2012).

Besides uniformly expressed regulators, other inputs into morphogen-regulated CREs also play an important role. These include transcriptional activators and inhibitors that are themselves morphogen target genes. An example of this is the Bcd target gene *Hunchback* (*Hb*). A CRE that regulates *Hb* depends on binding by Bcd and Zelda and the repressors Runt and Capicua (Chen et al. 2012), but in addition it also binds Hb itself (Fernandes et al. 2022). In this view, morphogen-responsive CREs act to integrate the different inputs to regulate gene expression. Since different CREs bind to different combinations of inputs, there is no consistent correlation between the binding affinity of the morphogen effector and the sensitivity of a specific target gene. For each gene it is the combination of positive and negative inputs that determines how the associated gene responds, and these inputs can affect the threshold sensitivity of a gene to morphogen input or properties such as the kinetics of gene induction (Dubrulle et al. 2015). Thus, CREs are responsible for the molecular implementation of the combinatorial logic of transcriptional regulation.

GENE REGULATORY NETWORKS IN MORPHOGEN INTERPRETATION

The transcriptional networks that result from the regulation of morphogen target genes by other morphogen target genes play a central role in spatial and temporal patterning (Davidson 2010). Even relatively simple transcriptional networks can produce complex gene expression dynamics

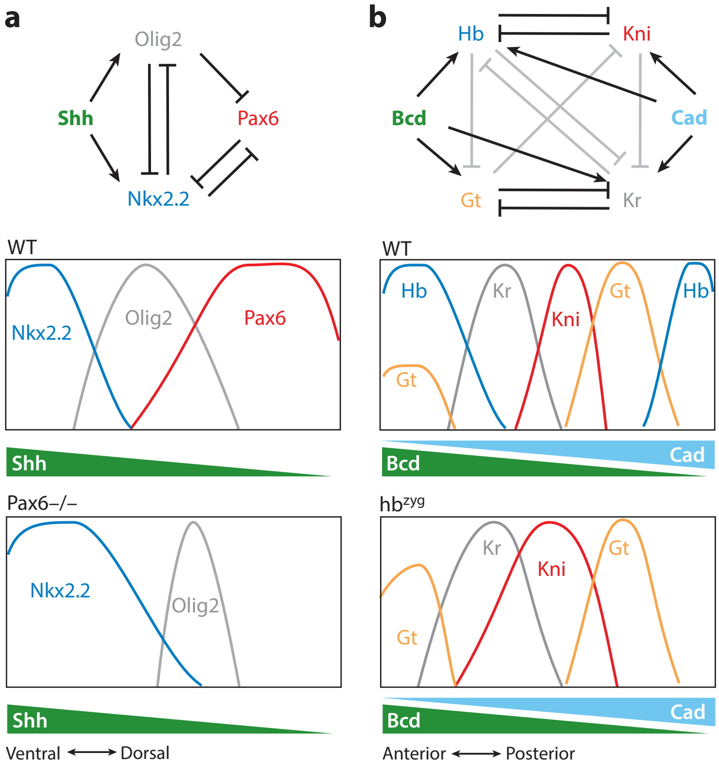

Figure 4

The positions of target gene boundaries are determined by the dynamics of morphogen-regulated GRNs. (*a*) In the vertebrate neural tube, a GRN sets up three ventral domains of gene expression (Nkx2.2, Olig2, and Pax6) in response to Shh signaling (*top*). In Pax6 mutants (*bottom*), the domains of Nkx2.2 and Olig2 expression shift along the dorsal-ventral axis compared to WT (*middle*), although the Shh morphogen gradient remains unchanged. (*b*) A GRN that sets up the boundaries of gap gene expression in the *Drosophila* embryo in response to Bcd and Cad gradients (*top*). Strong repressive interactions are shown in black and weak ones in gray. In zygotic hunchback mutants (*bottom*), the anterior domains of Gt, Kr, and Kni expression shift compared to WT (*middle*), although the Bcd gradient remains unchanged. Abbreviations: GRNs, gene regulatory networks; WT, wild type. Figure based on Jaeger (2011).

(Alon 2007). In silico studies of such small transcriptional subnetworks, comprising as few as two or three transcription factors, have identified several ways to generate morphogen-like spatial patterns (Cotterell & Sharpe 2010, Perez-Carrasco et al. 2018, Schaerli et al. 2014). The principles emerging from these studies are also observed in experimental analyses of real tissues.

Perhaps the two most studied examples of morphogen-regulated transcriptional networks are the anterior-posterior patterning of the *Drosophila* blastoderm and dorsal-ventral patterning of the neural tube. In both systems, networks of mutually inhibitory transcription factors expressed in neighboring domains create a series of bistable switches (Briscoe et al. 2000, Clyde et al. 2003, Ericson et al. 1997, Kraut & Levine 1991) (**Figure 4**). These allow cells to convert the graded input into a sharply delineated output to establish and stabilize all-or-nothing gene expression boundaries between cells with distinct regional identities. These transcriptional interactions influence the sensitivity of target genes to the morphogen and the position of gene expression boundaries. In the neural tube, the transcription factor Nkx2.2 is expressed in a region with high levels of

Shh signaling, adjacent to cells expressing another transcription factor, Pax6. In embryos lacking Pax6, Nkx2.2 is induced by lower levels of Shh and its expression expands, indicating that Pax6 represses Nkx2.2 and sets the boundary of Nkx2.2 expression (Balaskas et al. 2012, Ericson et al. 1997) (**Figure 4*a***). Similarly, in the *Drosophila* blastoderm, Hb restricts the expression of the transcription factor Knirps (Kni) (Clyde et al. 2003, Pankratz et al. 1992, Yu & Small 2008) (**Figure 4*b***). In mutants lacking Hb, Kni expression expands anteriorly into regions that have levels of Bcd that normally repress its expression (Jaeger 2011). These examples illustrate how transcriptional networks are crucial for morphogen interpretation, decoupling the absolute level of morphogen signaling from the positional identity adopted by cells.

The use of mathematical models, based on experimental data, has offered further insight into how the architecture and dynamics of transcriptional networks interpret morphogen gradients. In the *Drosophila* blastoderm, this analysis indicated that asymmetry in the strength of cross-repression between transcription factors expressed in adjacent regions leads to a cascade of feedback that sharpens and shifts the gene expression pattern relative to the Bcd gradient as development proceeds (Jaeger et al. 2004, Manu et al. 2009, Verd et al. 2018). In the neural tube, a transcriptional circuit comprising Shh-regulated transcription factors (the ACDC circuit) has been explored (Balaskas et al. 2012, Cohen et al. 2014, Panovska-Griffiths et al. 2013). In this case too, the cross-repressive interactions between the transcription factors play a central role in positioning the boundaries of gene expression in the tissue and account for the temporal sequence of gene expression observed in neural progenitors.

The neural tube gene regulatory network explains several additional features of patterning (Balaskas et al. 2012). Adaptation to Shh signaling in the neural tube generates a gradient of signaling levels that declines over time. The expression of downstream transcription factors is initiated at the early stages of neural tube development in a manner that depends on the levels of signaling. However, target gene expression boundaries do not correspond to constant signaling thresholds over time. This is because the initial state of the transcriptional network influences its subsequent dynamics: In this case, by initially triggering the repression of repressors, target genes are maintained in an active state. This effect is time dependent—signaling levels need to be maintained for long enough for repression to take effect. This explains both why the levels and duration of signaling matter for Shh-mediated patterning in the neural tube and why target genes are expressed in a temporal, not just spatial, order. Taken together, therefore, the Shh morphogen-controlled gene regulatory network is responsible for converting the continuous graded morphogen input into sharply delineated boundaries of gene expression, positioning these boundaries within the tissue, and integrating both the amount and duration of morphogen signaling into gene expression.

Three principles emerge (Briscoe & Small 2015). First, the morphogen provides a spatial cue to the tissue but does not determine positional identity alone. The level and duration of morphogen signaling are important, but these act together with tissue-specific transcription factors. Second, CREs associated with target genes integrate various inputs to control gene expression. Hence, CREs encode the logic of the gene regulatory network and set the activity of each gene. Third, the dynamics of the transcriptional network is responsible for generating sharp and stable patterns of genes and for correctly positioning the boundaries of gene expression within the tissue. In this view, the morphogen gradient provides the spatially polarized input into the system, but it is the downstream transcriptional network that interprets the gradient and establishes the patterning outcome.

One consequence of this view is that new analytical frameworks are needed to investigate the behavior of morphogen patterned systems. Most current modeling approaches consider morphogen gradient formation, signaling, and cellular response as separate constituents. This can mean that the function of the transcriptional network is treated as subordinate to the morphogen instead of as an integral part of the system. One approach is to combine tools from dynamical systems (Sáez et al. 2022) and control theory (Pezzotta & Briscoe 2022), which can accommodate morphogen spread, signaling, and response on an equal footing to develop analytical methods to dissect and understand the contribution of different parts of the system to the overall behavior. This could complement current methods aimed at explaining gene regulation by making morphogen gradient formation, cellular signaling, and transcription network integrated parts of a whole decision-making system.

PRECISION OF PATTERN FORMATION

Although molecular noise is a universal feature of biological processes (Raj & van Oudenaarden 2008, Raser & O'Shea 2005), morphogen-mediated patterning is remarkably reproducible and precise. For instance, along the anterior-posterior axis of the *Drosophila* blastoderm, the boundaries of gene expression are strikingly sharp and positioned with an accuracy of 99% or more (Petkova et al. 2019). This raises the question of how the precision of pattern formation is achieved.

A first step in addressing this question is to ask whether the morphogen gradient itself is sufficiently accurate to encode precise positional information for the generation of downstream patterning. This usually assumes direct information flow from extracellular morphogen to target gene expression and involves comparing the variation in morphogen concentration to the variation in gene expression at a particular position at a given time point (Tkačik & Gregor 2021). Spatiotemporal variability in morphogen production, spreading, and degradation gives rise to fluctuations in the morphogen concentration profiles between individuals. The specific mechanism and kinetics of morphogen gradient formation define the spatial profile of gradient imprecision. Typically, the uncertainty increases at large distances from the morphogen source and may be lowest at a specific set distance away from the source (Bollenbach et al. 2008). At the position of highest precision, morphogen gradients are generally found to be very precise, with positional uncertainty corresponding to less than three cell diameters (Bollenbach et al. 2008, Gregor et al. 2007a, Zagorski et al. 2017). The specific mechanism of gradient formation also determines the robustness of the mean morphogen concentration profile to fluctuations in the morphogen production rate. Mechanisms of gradient formation that maximize the gradient precision are not necessarily optimal for achieving robustness. Theoretical studies have suggested that complex pathway architecture (e.g., involving feedback on receptor production) could contribute to alleviating this trade-off and extend the range over which morphogen gradients can confer precise positional information, while at the same time being robust to fluctuations (Lo et al. 2015).

Additional patterning cues that provide independent input into target gene expression can also contribute to precision. The Bcd gradient is complemented by an antiparallel gradient consisting of the transcription factor Caudal from the posterior pole of the embryo (Macdonald & Struhl 1986, Mlodzik & Gehring 1987). Similarly, in the neural tube, the ventral gradient of Shh is matched by an antiparallel BMP signaling gradient from the dorsal neural tube (Barth et al. 1999, Mizutani et al. 2006, Zagorski et al. 2017). Theoretical analyses indicate that using information from antiparallel gradients can increase precision (Morishita & Iwasa 2009). One way that this can be achieved is through the downstream transcription network opposing gradients that induce the expression of opposing mutually repressing transcription factors (Manu et al. 2009, Sokolowski et al. 2012, Zagorski et al. 2017).

Features of the morphogen gene regulatory network can contribute to the precision of its patterning in various ways (Perez-Carrasco et al. 2016). For instance, having multiple redundant CREs for the same gene (so-called shadow enhancers) or having multiple alleles of the gene can help reduce randomness and increase the precision of gene expression (Cannavò et al. 2016, Perry et al. 2011). Additionally, the behavior of the gene regulatory network itself can impact gene expression accuracy. One well-studied example is *Hb*, which responds rapidly to form a sharp and precise boundary in the *Drosophila* blastoderm at a specific concentration of Bcd (Gregor et al. 2007a). This accuracy is achieved in part because Zelda and Hb also regulate *Hb* transcription and function with Bcd to ensure timely and precise expression (Fernandes et al. 2022). Similarly, deletion of a CRE in the neural tube regulatory network suggests the way in which the dynamics of gene expression produced by the gene regulatory network minimizes the effects of random fluctuations and contributes to the precision of gene expression (Exelby et al. 2021).

Finally, mechanisms downstream of the initial morphogen patterning, including differential adhesion, mechanical differences, and juxtacrine signaling, have been identified that correct errors and increase precision. Differential cell adhesion between cells with different identities can refine initially disordered patterns through cell sorting (Tsai et al. 2020, Xiong et al. 2013). Similarly, differences in Hedgehog signal transduction between cells in the anterior and posterior compartments of the fly wing disc contribute to the straightness of this boundary (Rudolf et al. 2015). By increasing the mechanical tension specifically at junctions of cells located between the anterior and posterior interface, cellular rearrangements that would result in cells intruding across the boundary are precluded, thus keeping the boundary straight.

MORPHOGENS AND THE REGULATION OF TISSUE GROWTH AND MORPHOGENESIS

Although their role in tissue patterning is extensively studied, morphogens also play a crucial role in cell survival and tissue growth in various tissues. In the developing limb bud, Shh has been shown to control both the proliferation and survival of mesenchyme cells (Zhu et al. 2008). Similarly, in the neural tube, Shh is necessary for cell survival and the formation of the correct tissue size (Chiang et al. 1996). In the *Drosophila* wing and eye discs, Dpp regulates tissue growth by adjusting the proliferation rate (Wartlick et al. 2011, 2014; Zecca et al. 1995). Several mechanisms have been suggested to explain this, including a mechanism in which Dpp levels need to be maintained above a threshold to inhibit the expression of the repressor Brinker (Barrio & Milán 2020, Bosch et al. 2017). Other studies indicate that cell division depends on the temporal changes in Dpp levels (Wartlick et al. 2011). Because the Dpp gradient scales with tissue size, the relative changes in signaling are uniform in space, translating the Dpp gradient into a spatially uniform proliferation rate. Nevertheless, Dpp expression outside of the stripe of producing cells along the anterior-posterior compartment boundary may be relevant for wing disc growth in some areas of the wing after a given developmental time point (Matsuda et al. 2021). Furthermore, Dpp is not the only signal that promotes growth in the wing disc. For instance, ecdysone signaling has been shown to be necessary for imaginal discs to respond to morphogens (Parker & Struhl 2020). These studies highlight the complexities of growth regulation and the challenge of determining the precise mechanism by which morphogens control growth. Progress will require methods to precisely modulate and measure signaling in space and time.

Morphogen signaling has also been shown to regulate cell behaviors that affect tissue morphogenesis. Some studies indicate that such roles for morphogen signaling are independent of cell identities. For example, in the zebrafish embryo, Nodal signaling specifies mesendoderm cell identities before the initiation of gastrulation movements (Hagos & Dougan 2007), while during gastrulation Nodal regulates the extent of protrusion formation and adhesiveness of mesendoderm cells (Pinheiro et al. 2022). This leads to the specification of a fraction of highly motile leader cells that can internalize beneath the ectodermal layer, pulling the remaining follower cells. Preferential adhesion between leaders and their followers ensures the orderly internalization of cells. FGF signaling gradients in the developing anterior-posterior body axis have also been linked to tissue morphogenesis and cell motility. The position of somite formation within the developing paraxial mesoderm is determined by FGF signaling, and it has also been suggested to control cell motility (Bénazéraf et al. 2010, Oginuma et al. 2017). Furthermore, BMP signaling in the zebrafish tail bud has been linked to the control of cell motion in the forming neural tube (Das et al. 2019). Together, these findings suggest that morphogens control cell mechanical properties in parallel with tissue patterning to ensure coordinated specification of cell fates and morphogenesis.

OUTLOOK

Recent years have seen considerable progress in understanding morphogen gradient formation and interpretation. Major questions remain, however (see the Future Issues). A variety of mechanisms, from the control of morphogen binding receptors to shuttling, trafficking, and growth, have evolved to regulate the spread of morphogens through tissues. An open question is to determine the quantitative contribution of each process to the spatiotemporal changes in the gradient shape. Studies that combine theoretical descriptions with quantitative assays of specific transport parameters at a subcellular scale are beginning to reveal how transport modes are integrated to determine gradient shape. Synthetic systems, which provide the means to engineer and manipulate specific scenarios, are also a powerful way to probe the logic of morphogen transport. Similarly, our understanding of how morphogens are interpreted to generate precise patterns of gene expression has advanced. Comparison between morphogens and tissues is revealing general principles, but quantitative models that incorporate these ideas are needed. There is increasing awareness of the role of morphogen signaling in coordinating growth and morphogenesis by controlling the cell cycle and mechanical properties of cells. This highlights the vital role of morphogens in determining tissue size and shape. However, challenges remain. Our ability to visualize directly and live image endogenous morphogen ligands and signaling remains limited in many model systems. This means that quantitative data are lacking, making it difficult to understand in detail how morphogen gradients form and control cellular responses. As our understanding of morphogen activity becomes increasingly fine grained, there is a need for sophisticated computational models that can integrate molecular, cellular, and tissue scales. Combining theory and experiment will provide deeper insight into the mechanisms and the underlying logic of the control of tissue development by morphogen gradients.

FUTURE ISSUES

1. While SDD appears to be the main mechanism for morphogen gradient formation, the molecular and cellular basis for this process is still unclear. In addition, the contribution of other mechanisms, including active transport and feedback, needs to be addressed. New quantitative live-imaging techniques will likely provide insight into these questions.
2. How do morphogen gradients change over time and how do these changes affect tissue patterning? Morphogen gradients are dynamic, yet the underlying mechanisms are not well understood. It is unclear whether different mechanisms apply to different morphogens and/or tissues. How such mechanisms contribute to gradient scaling with tissue size or contribute to the robustness of gradients to fluctuations in production or spread remains unclear.
3. What are the gene regulatory mechanisms that control the expression of genes responding directly to morphogens? What is the molecular and genomic basis for the control of target genes? How do the morphogen transcriptional effectors bring about

changes in transcription, and what role do other transcriptional regulators play? High-resolution quantitative data from new molecular and genomic tools will help answer these questions.

4. How do cells interpret morphogen signals to produce complex gene expression patterns? What are the mechanisms that convert graded morphogen input into specific changes in gene expression at precise spatial locations? What morphogen features, such as level, duration, or change, are important for pattern formation? New technologies such as single-cell transcriptomics will need to be complemented with new theoretical approaches to integrate and interpret increasingly complex data.
5. What are the sources and effects of biological noise in ligand spread, signal transduction, and gene regulation? Quantitative data and theoretical frameworks that provide multiscale descriptions of tissue patterning are needed to determine whether noise necessitates downstream correction mechanisms or whether noise is buffered by the system.
6. How is morphogen-controlled pattern formation integrated with growth and morphogenesis? Coordinating tissue patterning across different scales, from individual cells to whole organs, as well as in in vitro systems, requires an understanding of how morphogen signaling affects and is affected by cell proliferation, tissue growth, and mechanical forces. Investigating the role of these factors in tissue patterning and morphogenesis is an active area of research.

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LITERATURE CITED

Abu-Arish A, Porcher A, Czerwonka A, Dostatni N, Fradin C. 2010. High mobility of bicoid captured by fluorescence correlation spectroscopy: implication for the rapid establishment of its gradient. *Biophys. J.* 99(4):L33–35

Adler M, Alon U. 2018. Fold-change detection in biological systems. *Curr. Opin. Syst. Biol.* 8:81–89

Aguilar-Hidalgo D, Werner S, Wartlick O, González-Gaitán M, Friedrich BM, Jülicher F. 2018. Critical point in self-organized tissue growth. *Phys. Rev. Lett.* 120(19):198102

Aguirre-Tamaral A, Guerrero I. 2021. Improving the understanding of cytoneme-mediated morphogen gradients by in silico modeling. *PLOS Comput. Biol.* 17(8):e1009245

Akiyama T, Kamimura K, Firkus C, Takeo S, Shimmi O, Nakato H. 2008. Dally regulates Dpp morphogen gradient formation by stabilizing Dpp on the cell surface. *Dev. Biol.* 313(1):408–19

Alexandre C, Baena-Lopez A, Vincent J-P. 2014. Patterning and growth control by membrane-tethered Wingless. *Nature* 505(7482):180–85

Allen BL, Tenzen T, McMahon AP. 2007. The Hedgehog-binding proteins Gas1 and Cdo cooperate to positively regulate Shh signaling during mouse development. *Genes Dev*. 21(10):1244–57

Almuedo-Castillo M, Bläßle A, Mörsdorf D, Marcon L, Soh GH, et al. 2018. Scale-invariant patterning by size-dependent inhibition of Nodal signalling. *Nat. Cell Biol.* 20(9):1032–42

Alon U. 2007. Network motifs: theory and experimental approaches. *Nat. Rev. Genet.* 8(6):450–61

Athilingam T, Nelanuthala AVS, Breen C, Wohland T, Saunders TE. 2022. Long-ranged formation of the Bicoid gradient requires multiple dynamic modes that spatially vary across the embryo. bioRxiv 2022.09.28.509874. **https://doi.org/10.1101/2022.09.28**

Averbukh I, Ben-Zvi D, Mishra S, Barkai N. 2014. Scaling morphogen gradients during tissue growth by a cell division rule. *Development* 141(10):2150–56

Balaskas N, Ribeiro A, Panovska J, Dessaud E, Sasai N, et al. 2012. Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell* 148(1–2):273–84

Barolo S, Posakony JW. 2002. Three habits of highly effective signaling pathways: principles of transcriptional control by developmental cell signaling. *Genes Dev*. 16(10):1167–81

Barrio L, Milán M. 2020. Regulation of anisotropic tissue growth by two orthogonal signaling centers. *Dev. Cell* 52:659–72.e3

Barth KA, Kishimoto Y, Rohr KB, Seydler C, Schulte-Merker S, Wilson SW. 1999. Bmp activity establishes a gradient of positional information throughout the entire neural plate. *Development* 126(22):4977–87

Bénazéraf B, Francois P, Baker RE, Denans N, Little CD, Pourquié O. 2010. A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. *Nature* 466(7303):248–52

Ben-Zvi D, Barkai N. 2010. Scaling of morphogen gradients by an expansion-repression integral feedback control. *PNAS* 107(15):6924–29

Ben-Zvi D, Pyrowolakis G, Barkai N, Shilo B-Z. 2011. Expansion-repression mechanism for scaling the Dpp activation gradient in *Drosophila* wing imaginal discs. *Curr. Biol.* 21(16):1391–96

Ben-Zvi D, Shilo B-Z, Fainsod A, Barkai N. 2008. Scaling of the BMP activation gradient in *Xenopus* embryos. *Nature* 453:1205–11

Berezhkovskii AM, Sample C, Shvartsman SY. 2010. How long does it take to establish a morphogen gradient? *Biophysical J*. 99(8):L59–61

Bergmann S, Sandler O, Sberro H, Shnider S, Schejter E, et al. 2007. Pre-steady-state decoding of the Bicoid morphogen gradient. *PLOS Biol*. 5(2):e46

Bergsland M, Ramsköld D, Zaouter C, Klum S, Sandberg R, Muhr J. 2011. Sequentially acting Sox transcription factors in neural lineage development. *Genes Dev*. 25(23):2453–64

Bernheim S, Meilhac SM. 2020. Mesoderm patterning by a dynamic gradient of retinoic acid signalling. *Philos. Trans. R. Soc. B Biol. Sci.* 375(1809):20190556

Bischoff M, Gradilla A-C, Seijo I, Andrés G, Rodríguez-Navas C, et al. 2013. Cytonemes are required for the establishment of a normal Hedgehog morphogen gradient in *Drosophila* epithelia. *Nat. Cell Biol.* 15(11):1269–81

Bittig T, Wartlick O, González-Gaitán M, Jülicher F. 2009. Quantification of growth asymmetries in developing epithelia. *Eur. Phys. J. E* 30(1):93–99

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, et al. 2005. The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature* 433(7021):39–44

Bökel C, Schwabedissen A, Entchev E, Renaud O, González-Gaitán M. 2006. Sara endosomes and the maintenance of Dpp signaling levels across mitosis. *Science* 314(5802):1135–39

Bollenbach T, Kruse K, Pantazis P, González-Gaitán M, Jülicher F. 2005. Robust formation of morphogen gradients. *Phys. Rev. Lett.* 94(1):018103

Bollenbach T, Kruse K, Pantazis P, González-Gaitán M, Jülicher F. 2007. Morphogen transport in epithelia. *Phys. Rev. E* 75(1):011901

Bollenbach T, Pantazis P, Kicheva A, Bökel C, González-Gaitán M, Jülicher F. 2008. Precision of the Dpp gradient. *Development* 135(6):1137–46

Bornemann DJ, Duncan JE, Staatz W, Selleck S, Warrior R. 2004. Abrogation of heparan sulfate synthesis in *Drosophila* disrupts the Wingless, Hedgehog and Decapentaplegic signaling pathways. *Development* 131(9):1927–38

Bosch PS, Ziukaite R, Alexandre C, Basler K, Vincent J-P. 2017. Dpp controls growth and patterning in Drosophila wing precursors through distinct modes of action. *eLife* 6:e22546

Briscoe J, Chen Y, Jessell TM, Struhl G. 2001. A Hedgehog-insensitive form of patched provides evidence for direct long-range morphogen activity of Sonic Hedgehog in the neural tube. *Mol. Cell* 7(6):1279–91

Briscoe J, Pierani A, Jessell TM, Ericson J. 2000. A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* 101(4):435–45

Briscoe J, Small S. 2015. Morphogen rules: design principles of gradient-mediated embryo patterning. *Development* 142(23):3996–4009

Brunt L, Greicius G, Rogers S, Evans BD, Virshup DM, et al. 2021. Vangl2 promotes the formation of long cytonemes to enable distant Wnt/β-catenin signaling. *Nat. Commun.* 12(1):2058

Burke R, Nellen D, Bellotto M, Hafen E, Senti KA, et al. 1999. Dispatched, a novel sterol-sensing domain protein dedicated to the release of cholesterol-modified Hedgehog from signaling cells. *Cell* 99(7):803–15

Cadigan KM, Fish MP, Rulifson EJ, Nusse R. 1998. Wingless repression of *Drosophila frizzled 2* expression shapes the Wingless morphogen gradient in the wing. *Cell* 93(5):767–77

Camacho-Aguilar E, Yoon S, Ortiz-Salazar MA, Warmflash A. 2022. Combinatorial interpretation of BMP and WNT allows BMP to act as a morphogen in time but not in concentration. bioRxiv 2022.11.11.516212. **https://doi.org/10.1101/2022.11.11.516212**

Cannavò E, Khoueiry P, Garfield DA, Geeleher P, Zichner T, et al. 2016. Shadow enhancers are pervasive features of developmental regulatory networks. *Curr. Biol.* 26(1):38–51

Čapek D, Müller P. 2019. Positional information and tissue scaling during development and regeneration. *Development* 146(24):dev177709

Cardin AD, Weintraub HJ. 1989. Molecular modeling of protein-glycosaminoglycan interactions. *Arteriosclerosis* 9(1):21–32

Caspary T, García-García MJ, Huangfu D, Eggenschwiler JT, Wyler MR, et al. 2002. Mouse dispatched homolog1 is required for long-range, but not juxtacrine, Hh signaling. *Curr. Biol.* 12(18):1628–32

Chang Y-H, Sun YH. 2014. Carrier of Wingless (Cow), a secreted heparan sulfate proteoglycan, promotes extracellular transport of Wingless. *PLOS ONE* 9(10):e111573

Chen H, Xu Z, Mei C, Yu D, Small S. 2012. A system of repressor gradients spatially organizes the boundaries of Bicoid-dependent target genes. *Cell* 149(3):618–29

Chen M-H, Li Y-J, Kawakami T, Xu S-M, Chuang P-T. 2004. Palmitoylation is required for the production of a soluble multimeric Hedgehog protein complex and long-range signaling in vertebrates. *Genes Dev.* 18(6):641–59

Cheung D, Miles C, Kreitman M, Ma J. 2011. Scaling of the Bicoid morphogen gradient by a volume-dependent production rate. *Development* 138(13):2741–49

Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, et al. 1996. Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* 383(6599):407–13

Clyde DE, Corado MSG, Wu X, Paré A, Papatsenko D, Small S. 2003. A self-organizing system of repressor gradients establishes segmental complexity in *Drosophila*. *Nature* 426(6968):849–53

Cohen M, Page KM, Perez-Carrasco R, Barnes CP, Briscoe J. 2014. A theoretical framework for the regulation of Shh morphogen-controlled gene expression. *Development* 141(20):3868–78

Cohen-Saidon C, Cohen AA, Sigal A, Liron Y, Alon U. 2009. Dynamics and variability of ERK2 response to EGF in individual living cells. *Mol. Cell* 36(5):885–93

Collins ZM, Ishimatsu K, Tsai TYC, Megason SG. 2018. A Scube2-Shh feedback loop links morphogen release to morphogen signaling to enable scale invariant patterning of the ventral neural tube. bioRxiv 469239. **https://doi.org/10.1101/469239**

Cotterell J, Sharpe J. 2010. An atlas of gene regulatory networks reveals multiple three-gene mechanisms for interpreting morphogen gradients. *Mol. Syst. Biol.* 6:425

Dalessi S, Neves A, Bergmann S. 2012. Modeling morphogen gradient formation from arbitrary realistically shaped sources. *J. Theor. Biol.* 294:130–38

Das D, Jülich D, Schwendinger-Schreck J, Guillon E, Lawton AK, et al. 2019. Organization of embryonic morphogenesis via mechanical information. *Dev. Cell* 49(6):829–39.e5

Davidson EH. 2010. Emerging properties of animal gene regulatory networks. *Nature* 468(7326):911–20

Dessaud E, Ribes V, Balaskas N, Yang LL, Pierani A, et al. 2010. Dynamic assignment and maintenance of positional identity in the ventral neural tube by the morphogen Sonic hedgehog. *PLOS Biol.* 8(6):e1000382

Dessaud E, Yang LL, Hill K, Cox B, Ulloa F, et al. 2007. Interpretation of the sonic hedgehog morphogen gradient by a temporal adaptation mechanism. *Nature* 450(7170):717–20

Dickmann JEM, Rink JC, Jülicher F. 2022. Long-range morphogen gradient formation by cell-to-cell signal propagation. *Phys. Biol.* 19(6):066001

Driever W, Nüsslein-Volhard C. 1988. The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* 54(1):95–104

Driever W, Thoma G, Nüsslein-Volhard C. 1989. Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature* 340(6232):363–67

Drocco JA, Grimm O, Tank DW, Wieschaus E. 2011. Measurement and perturbation of morphogen lifetime: effects on gradient shape. *Biophys. J.* 101(8):1807–15

Du L, Sohr A, Yan G, Roy S. 2018. Feedback regulation of cytoneme-mediated transport shapes a tissue-specific FGF morphogen gradient. *eLife* 7:e38137

Dubrulle J, Jordan BM, Akhmetova L, Farrell JA, Kim S-H, et al. 2015. Response to Nodal morphogen gradient is determined by the kinetics of target gene induction. *eLife* 4:e05042

Dubrulle J, Pourquié O. 2004. *fgf8* mRNA decay establishes a gradient that couples axial elongation to patterning in the vertebrate embryo. *Nature* 427(6973):419–22

Durrieu L, Kirrmaier D, Schneidt T, Kats I, Raghavan S, et al. 2018. Bicoid gradient formation mechanism and dynamics revealed by protein lifetime analysis. *Mol. Syst. Biol.* 14(9):e8355

Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, et al. 1993. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* 75(7):1417–30

Eldar A, Dorfman R, Weiss D, Ashe H, Shilo B-Z, Barkai N. 2002. Robustness of the BMP morphogen gradient in *Drosophila* embryonic patterning. *Nature* 419(6904):304–8

Eldar A, Rosin D, Shilo B-Z, Barkai N. 2003. Self-enhanced ligand degradation underlies robustness of morphogen gradients. *Dev. Cell* 5(4):635–46

Ericson J, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, et al. 1997. Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. *Cell* 90(1):169–80

Exelby K, Herrera-Delgado E, Perez LG, Perez-Carrasco R, Sagner A, et al. 2021. Precision of tissue patterning is controlled by dynamical properties of gene regulatory networks. *Development* 148(4):dev197566

Fernandes G, Tran H, Andrieu M, Diaw Y, Perez Romero C, et al. 2022. Synthetic reconstruction of the *hunchback* promoter specifies the role of Bicoid, Zelda and Hunchback in the dynamics of its transcription. *eLife* 11:e74509

Foo SM, Sun Y, Lim B, Ziukaite R, O'Brien K, et al. 2014. Zelda potentiates morphogen activity by increasing chromatin accessibility. *Curr. Biol.* 24(12):1341–46

Fried P, Iber D. 2014. Dynamic scaling of morphogen gradients on growing domains. *Nat. Commun.* 5(1):5077

Fujise M, Takeo S, Kamimura K, Matsuo T, Aigaki T, et al. 2003. Dally regulates Dpp morphogen gradient formation in the *Drosophila* wing. *Development* 130(8):1515–22

Fulton T, Spiess K, Thomson L, Wang Y, Clark B, et al. 2021. Cell rearrangement generates pattern emergence as a function of temporal morphogen exposure. bioRxiv 2021.02.05.429898. **https://doi.org/10.1101/2021.02.05.429898**

Fulton T, Verd B, Steventon B. 2022. The unappreciated generative role of cell movements in pattern formation. *R. Soc. Open Sci.* 9(4):211293

Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, et al. 2007. PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. *Nature* 449(7165):1053–57

Goentoro L, Kirschner MW. 2009. Evidence that fold-change, and not absolute level, of β-catenin dictates Wnt signaling. *Mol. Cell* 36(5):872–84

Gorfinkiel N, Sierra J, Callejo A, Ibañez C, Guerrero I. 2005. The *Drosophila* ortholog of the human Wnt inhibitor factor *Shifted* controls the diffusion of lipid-modified Hedgehog. *Dev. Cell* 8(2):241–53

Green JBA, Sharpe J. 2015. Positional information and reaction-diffusion: Two big ideas in developmental biology combine. *Development* 142(7):1203–11

Greenfeld H, Lin J, Mullins MC. 2021. The BMP signaling gradient is interpreted through concentration thresholds in dorsal-ventral axial patterning. *PLOS Biol.* 19(1):e3001059

Gregor T, Bialek W, de Ruyter van Steveninck RR, Tank DW, Wieschaus EF. 2005. Diffusion and scaling during early embryonic pattern formation. *PNAS* 102(51):18403–7

Gregor T, Tank DW, Wieschaus EF, Bialek W. 2007a. Probing the limits to positional information. *Cell* 130(1):153–64

Gregor T, Wieschaus EF, McGregor AP, Bialek W, Tank DW. 2007b. Stability and nuclear dynamics of the Bicoid morphogen gradient. *Cell* 130(1):141–52

Grieneisen VA, Xu J, Marée AFM, Hogeweg P, Scheres B. 2007. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449(7165):1008–13

Gritsman K, Talbot WS, Schier AF. 2000. Nodal signaling patterns the organizer. *Development* 127:921–32

Gross JC, Chaudhary V, Bartscherer K, Boutros M. 2012. Active Wnt proteins are secreted on exosomes. *Nat. Cell Biol.* 14(10):1036–45

Häcker U, Lin X, Perrimon N. 1997. The *Drosophila* sugarless gene modulates Wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis. *Development* 124(18):3565–73

Haerry TE, Heslip TR, Marsh JL, O'Connor MB. 1997. Defects in glucuronate biosynthesis disrupt Wingless signaling in *Drosophila*. *Development* 124(16):3055–64

Hagos EG, Dougan ST. 2007. Time-dependent patterning of the mesoderm and endoderm by Nodal signals in zebrafish. *BMC Dev. Biol.* 7:22

Hamaratoglu F, de Lachapelle AM, Pyrowolakis G, Bergmann S, Affolter M. 2011. Dpp signaling activity requires Pentagone to scale with tissue size in the growing *Drosophila* wing imaginal disc. *PLOS Biol.* 9(10):e1001182

Harish RK, Gupta M, Zöller D, Hartmann H, Gheisari A, et al. 2022. Real-time monitoring of endogenous Fgf8a gradient attests to its role as a morphogen during zebrafish gastrulation. bioRxiv 2022.04.26.488902. **https://doi.org/10.1101/2022.04.26.488902**

Harrison MM, Li X-Y, Kaplan T, Botchan MR, Eisen MB. 2011. Zelda binding in the early *Drosophila melanogaster* embryo marks regions subsequently activated at the maternal-to-zygotic transition. *PLOS Genet.* 7(10):e1002266

Hollway GE, Maule J, Gautier P, Evans TM, Keenan DG, et al. 2006. Scube2 mediates Hedgehog signalling in the zebrafish embryo. *Dev. Biol.* 294(1):104–18

Hsieh JC, Kodjabachian L, Rebbert ML, Rattner A, Smallwood PM, et al. 1999. A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* 398(6726):431–36

Hu Q, Ueno N, Behringer RR. 2004. Restriction of BMP4 activity domains in the developing neural tube of the mouse embryo. *EMBO Rep.* 5(7):734–39

Huang A, Amourda C, Zhang S, Tolwinski NS, Saunders TE. 2017. Decoding temporal interpretation of the morphogen Bicoid in the early *Drosophila* embryo. *eLife* 6:e26258

Huang CY, Ferrell JE. 1996. Ultrasensitivity in the mitogen-activated protein kinase cascade. *PNAS* 93(19):10078–83

Jaeger J. 2011. The gap gene network. *Cell. Mol. Life Sci.* 68(2):243–74

Jaeger J, Surkova S, Blagov M, Janssens H, Kosman D, et al. 2004. Dynamic control of positional information in the early *Drosophila* embryo. *Nature* 430(6997):368–71

Jeong J, McMahon AP. 2005. Growth and pattern of the mammalian neural tube are governed by partially overlapping feedback activities of the hedgehog antagonists patched 1 and Hhip1. *Development* 132(1):143–54

Jiang J, Levine M. 1993. Binding affinities and cooperative interactions with bHLH activators delimit threshold responses to the dorsal gradient morphogen. *Cell* 72(5):741–52

Jullien J, Gurdon J. 2005. Morphogen gradient interpretation by a regulated trafficking step during ligand-receptor transduction. *Genes Dev.* 19(22):2682–94

Kanodia JS, Liang H-L, Kim Y, Lim B, Zhan M, et al. 2012. Pattern formation by graded and uniform signals in the early *Drosophila* embryo. *Biophys. J.* 102(3):427–33

Kawakami A, Nojima Y, Toyoda A, Takahoko M, Satoh M, et al. 2005. The zebrafish-secreted matrix protein you/scube2 is implicated in long-range regulation of hedgehog signaling. *Curr. Biol.* 15(5):480–88

Kawakami T, Kawcak T, Li Y-J, Zhang W, Hu Y, Chuang P-T. 2002. Mouse dispatched mutants fail to distribute hedgehog proteins and are defective in hedgehog signaling. *Development* 129(24):5753–65

Kicheva A, Bollenbach T, Ribeiro A, Valle HP, Lovell-Badge R, et al. 2014. Coordination of progenitor specification and growth in mouse and chick spinal cord. *Science* 345(6204):1254927

Kicheva A, Bollenbach T, Wartlick O, Jülicher F, Gonzalez-Gaitan M. 2012. Investigating the principles of morphogen gradient formation: from tissues to cells. *Curr. Opin. Genet. Dev.* 22(6):527–32

Kicheva A, Pantazis P, Bollenbach T, Kalaidzidis Y, Bittig T, et al. 2007. Kinetics of morphogen gradient formation. *Science* 315(5811):521–25

Klein T, Arias AM. 1998. Different spatial and temporal interactions between *Notch*, *wingless*, and *vestigial* specify proximal and distal pattern elements of the wing in *Drosophila*. *Dev. Biol.* 194(2):196–212

Korkut C, Ataman B, Ramachandran P, Ashley J, Barria R, et al. 2009. *Trans*-synaptic transmission of vesicular Wnt signals through Evi/Wntless. *Cell* 139(2):393–404

Kornberg TB. 2017. Distributing signaling proteins in space and time: the province of cytonemes. *Curr. Opin. Genet. Dev.* 45:22–27

Kraut R, Levine M. 1991. Mutually repressive interactions between the gap genes *giant* and *Krüppel* define middle body regions of the *Drosophila* embryo. *Development* 111(2):611–21

Kuhn T, Landge AN, Mörsdorf D, Coßmann J, Gerstenecker J, et al. 2022. Single-molecule tracking of Nodal and Lefty in live zebrafish embryos supports hindered diffusion model. *Nat. Commun.* 13(1):6101

Lander AD, Lo W-C, Nie Q, Wan FYM. 2009. The measure of success: constraints, objectives, and tradeoffs in morphogen-mediated patterning. *Cold Spring Harb. Perspect. Biol.* 1(1):a002022

Lecuit T, Cohen SM. 1998. Dpp receptor levels contribute to shaping the Dpp morphogen gradient in the *Drosophila* wing imaginal disc. *Development* 125(24):4901–7

Leyns L, Bouwmeester T, Kim SH, Piccolo S, De Robertis EM. 1997. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. *Cell* 88(6):747–56

Li L, Wang X, Chai J, Wang X, Buganza-Tepole A, Umulis DM. 2022. Determining the role of advection in patterning by bone morphogenetic proteins through neural network model-based acceleration of a 3D finite element model of the zebrafish embryo. *Front. Syst. Biol.* 2:983372

Li P, Markson JS, Wang S, Chen S, Vachharajani V, Elowitz MB. 2018. Morphogen gradient reconstitution reveals Hedgehog pathway design principles. *Science* 360(6388):543–48

Li X-Y, Eisen MB. 2018. Zelda potentiates transcription factor binding to zygotic enhancers by increasing local chromatin accessibility during early *Drosophila melanogaster* embryogenesis. bioRxiv 380857. **https://doi.org/10.1101/380857**

Liégeois S, Benedetto A, Garnier J-M, Schwab Y, Labouesse M. 2006. The V0-ATPase mediates apical secretion of exosomes containing Hedgehog-related proteins in *Caenorhabditis elegans*. *J. Cell Biol.* 173(6):949–61

Little SC, Tkačik G, Kneeland TB, Wieschaus EF, Gregor T. 2011. The formation of the Bicoid morphogen gradient requires protein movement from anteriorly localized mRNA. *PLOS Biol.* 9(3):e1000596

Liu L, Nemashkalo A, Rezende L, Jung JY, Chhabra S, et al. 2022. Nodal is a short-range morphogen with activity that spreads through a relay mechanism in human gastruloids. *Nat. Commun.* 13(1):497

Lo W-C, Zhou S, Wan FY-M, Lander AD, Nie Q. 2015. Robust and precise morphogen-mediated patterning: trade-offs, constraints and mechanisms. *J. R. Soc. Interface* 12(102):20141041

Lord ND, Carte AN, Abitua PB, Schier AF. 2021. The pattern of nodal morphogen signaling is shaped by co-receptor expression. *eLife* 10:e54894

Ma Y, Erkner A, Gong R, Yao S, Taipale J, et al. 2002. Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of dispatched. *Cell* 111(1):63–75

Macdonald PM, Struhl G. 1986. A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. *Nature* 324:537–45

Mähönen AP, Ten Tusscher K, Siligato R, Smetana O, Díaz-Triviño S, et al. 2014. PLETHORA gradient formation mechanism separates auxin responses. *Nature* 515(7525):125–29

Manu Surkova S, Spirov AV, Gursky VV, Janssens H, et al. 2009. Canalization of gene expression in the *Drosophila* blastoderm by gap gene cross regulation. *PLOS Biol*. 7(3):e1000049

Mateus R, Holtzer L, Seum C, Hadjivasiliou Z, Dubois M, et al. 2020. BMP signaling gradient scaling in the zebrafish pectoral fin. *Cell Rep*. 30(12):4292–302.e7

Matsuda S, Schaefer JV, Mii Y, Hori Y, Bieli D, et al. 2021. Asymmetric requirement of Dpp/BMP morphogen dispersal in the *Drosophila* wing disc. *Nat. Commun.* 12:6435

Mattes B, Dang Y, Greicius G, Kaufmann LT, Prunsche B, et al. 2018. Wnt/PCP controls spreading of Wnt/β-catenin signals by cytonemes in vertebrates. *eLife* 7:e36953

Matusek T, Wendler F, Polès S, Pizette S, D'Angelo G, et al. 2014. The ESCRT machinery regulates the secretion and long-range activity of Hedgehog. *Nature* 516(7529):99–103

McGough IJ, Vecchia L, Bishop B, Malinauskas T, Beckett K, et al. 2020. Glypicans shield the Wnt lipid moiety to enable signalling at a distance. *Nature* 585(7823):85–90

Melen GJ, Levy S, Barkai N, Shilo B-Z. 2005. Threshold responses to morphogen gradients by zero-order ultrasensitivity. *Mol. Syst. Biol.* 1:2005.0028

Merino MM, Seum C, Dubois M, Gonzalez-Gaitan M. 2022. A role for Flower and cell death in controlling morphogen gradient scaling. *Nat. Cell Biol.* 24(4):424–33

Míguez DG, Iannini A, García-Morales D, Casares F. 2022. The effects of Hh morphogen source movement on signaling dynamics. *Development* 149(23):dev199842

Mihara E, Hirai H, Yamamoto H, Tamura-Kawakami K, Matano M, et al. 2016. Active and water-soluble form of lipidated Wnt protein is maintained by a serum glycoprotein afamin/α-albumin. *eLife* 5:e11621

Mir M, Stadler MR, Ortiz SA, Hannon CE, Harrison MM, et al. 2018. Dynamic multifactor hubs interact transiently with sites of active transcription in *Drosophila* embryos. *eLife* 7:e40497

Mizutani CM, Meyer N, Roelink H, Bier E. 2006. Threshold-dependent BMP-mediated repression: a model for a conserved mechanism that patterns the neuroectoderm. *PLOS Biol.* 4(10):e313

Mlodzik M, Gehring WJ. 1987. Expression of the *caudal* gene in the germ line of Drosophila: formation of an RNA and protein gradient during early embryogenesis. *Cell* 48(3):465–78

Morishita Y, Iwasa Y. 2009. Accuracy of positional information provided by multiple morphogen gradients with correlated noise. *Phys. Rev. E Stat. Nonlin. Soft. Matter Phys.* 79(6, Part 1):061905

Müller P, Rogers KW, Jordan BM, Lee JS, Robson D, et al. 2012. Differential diffusivity of Nodal and Lefty underlies a reaction-diffusion patterning system. *Science* 336(6082):721–24

Müller P, Rogers KW, Yu SR, Brand M, Schier AF. 2013. Morphogen transport. *Development* 140(8):1621–38

Mulligan KA, Fuerer C, Ching W, Fish M, Willert K, Nusse R. 2012. Secreted Wingless-interacting molecule (Swim) promotes long-range signaling by maintaining Wingless solubility. *PNAS* 109(2):370–77

Nakato H, Li J-P. 2016. Functions of heparan sulfate proteoglycans in development: insights from *Drosophila* models. *Int. Rev. Cell Mol. Biol.* 325:275–93

Nugent MA, Edelman ER. 1992. Kinetics of basic fibroblast growth factor binding to its receptor and heparan sulfate proteoglycan: a mechanism for cooperactivity. *Biochemistry* 31(37):8876–83

Ochoa-Espinosa A, Yucel G, Kaplan L, Pare A, Pura N, et al. 2005. The role of binding site cluster strength in Bicoid-dependent patterning in *Drosophila*. *PNAS* 102(14):4960–65

Oginuma M, Moncuquet P, Xiong F, Karoly E, Chal J, et al. 2017. A gradient of glycolytic activity coordinates FGF and Wnt signaling during elongation of the body axis in amniote embryos. *Dev. Cell* 40(4):342–53.e10

Ohkawara B, Iemura S, ten Dijke P, Ueno N. 2002. Action range of BMP is defined by its N-terminal basic amino acid core. *Curr. Biol.* 12(3):205–9

Oosterveen T, Kurdija S, Alekseenko Z, Uhde CW, Bergsland M, et al. 2012. Mechanistic differences in the transcriptional interpretation of local and long-range Shh morphogen signaling. *Dev. Cell* 23(5):1006–19

Panáková D, Sprong H, Marois E, Thiele C, Eaton S. 2005. Lipoprotein particles are required for Hedgehog and Wingless signalling. *Nature* 435(7038):58–65

Pankratz MJ, Busch M, Hoch M, Seifert E, Jäckle H. 1992. Spatial control of the gap gene *knirps* in the *Drosophila* embryo by posterior morphogen system. *Science* 255(5047):986–89

Panovska-Griffiths J, Page KM, Briscoe J. 2013. A gene regulatory motif that generates oscillatory or multiway switch outputs. *J. R. Soc. Interface* 10(79):20120826

Parker J, Struhl G. 2020. Control of *Drosophila* wing size by morphogen range and hormonal gating. *PNAS* 117(50):31935–44

Patel A, Wu Y, Han X, Su Y, Maugel T, et al. 2022. Cytonemes coordinate asymmetric signaling and organization in the *Drosophila* muscle progenitor niche. *Nat. Commun.* 13(1):1185

Perez-Carrasco R, Barnes CP, Schaerli Y, Isalan M, Briscoe J, Page KM. 2018. Combining a toggle switch and a repressilator within the AC-DC circuit generates distinct dynamical behaviors. *Cell Syst*. 6(4):521–30.e3

Perez-Carrasco R, Guerrero P, Briscoe J, Page KM. 2016. Intrinsic noise profoundly alters the dynamics and steady state of morphogen-controlled bistable genetic switches. *PLOS Comput. Biol.* 12(10):e1005154

Perry MW, Boettiger AN, Levine M. 2011. Multiple enhancers ensure precision of gap gene-expression patterns in the *Drosophila* embryo. *PNAS* 108(33):13570–75

Peterson KA, Nishi Y, Ma W, Vedenko A, Shokri L, et al. 2012. Neural-specific Sox2 input and differential Gli-binding affinity provide context and positional information in Shh-directed neural patterning. *Genes Dev*. 26(24):2802–16

Petkova MD, Tkačik G, Bialek W, Wieschaus EF, Gregor T. 2019. Optimal decoding of cellular identities in a genetic network. *Cell* 176(4):844–55.e15

Pezzotta A, Briscoe J. 2022. Optimal control of gene regulatory networks for morphogen-driven tissue patterning. bioRxiv 2022.07.26.501519. **https://doi.org/10.1101/2022.07.26.501519**

Pfeiffer S, Alexandre C, Calleja M, Vincent JP. 2000. The progeny of *wingless*-expressing cells deliver the signal at a distance in *Drosophila* embryos. *Curr. Biol.* 10(6):321–24

Pinheiro D, Kardos R, Hannezo É, Heisenberg C-P. 2022. Morphogen gradient orchestrates pattern-preserving tissue morphogenesis via motility-driven unjamming. *Nat. Phys.* 18(12):1482–93

Pomreinke AP, Soh GH, Rogers KW, Bergmann JK, Bläßle AJ, Müller P. 2017. Dynamics of BMP signaling and distribution during zebrafish dorsal-ventral patterning. *eLife* 6:e25861

Rahimi N, Carmon S, Averbukh I, Khajouei F, Sinha S, et al. 2020. Global shape of Toll activation is determined by *wntD* enhancer properties. *PNAS* 117(3):1552–58

Raj A, van Oudenaarden A. 2008. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* 135(2):216–26

Ramel M-C, Hill CS. 2013. The ventral to dorsal BMP activity gradient in the early zebrafish embryo is determined by graded expression of BMP ligands. *Dev. Biol.* 378(2):170–82

Ramírez-Weber FA, Kornberg TB. 1999. Cytonemes: cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell* 97(5):599–607

Raser JM, O'Shea EK. 2005. Noise in gene expression: origins, consequences, and control. *Science* 309(5743):2010–13

Rasolonjanahary M, Vasiev B. 2016. Scaling of morphogenetic patterns in reaction-diffusion systems. *J. Theor. Biol.* 404:109–19

Romanova-Michaelides M, Hadjivasiliou Z, Aguilar-Hidalgo D, Basagiannis D, Seum C, et al. 2022. Morphogen gradient scaling by recycling of intracellular Dpp. *Nature* 602(7896):287–93

Roy S, Huang H, Liu S, Kornberg TB. 2014. Cytoneme-mediated contact-dependent transport of the *Drosophila* decapentaplegic signaling protein. *Science* 343(6173):1244624

Rudolf K, Umetsu D, Aliee M, Sui L, Jülicher F, Dahmann C. 2015. A local difference in Hedgehog signal transduction increases mechanical cell bond tension and biases cell intercalations along the *Drosophila* anteroposterior compartment boundary. *Development* 142(22):3845–58

Sáez M, Blassberg R, Camacho-Aguilar E, Siggia ED, Rand DA, Briscoe J. 2022. Statistically derived geometrical landscapes capture principles of decision-making dynamics during cell fate transitions. *Cell Syst*. 13(1):12–28.e3

Sako K, Pradhan SJ, Barone V, Inglés-Prieto Á, Müller P, et al. 2016. Optogenetic control of nodal signaling reveals a temporal pattern of nodal signaling regulating cell fate specification during gastrulation. *Cell Rep*. 16(3):866–77

Sanders TA, Llagostera E, Barna M. 2013. Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. *Nature* 497(7451):628–32

Schaerli Y, Munteanu A, Gili M, Cotterell J, Sharpe J, Isalan M. 2014. A unified design space of synthetic stripe-forming networks. *Nat. Commun.* 5:4905

Shimizu K, Gurdon JB. 1999. A quantitative analysis of signal transduction from activin receptor to nucleus and its relevance to morphogen gradient interpretation. *PNAS* 96(12):6791–96

Shimmi O, Umulis D, Othmer H, O'Connor MB. 2005. Facilitated transport of a Dpp/Scw heterodimer by Sog/Tsg leads to robust patterning of the *Drosophila* blastoderm embryo. *Cell* 120(6):873–86

Shvartsman SY, Baker RE. 2012. Mathematical models of morphogen gradients and their effects on gene expression: mathematical models of morphogen gradients. *WIREs Dev. Biol.* 1(5):715–30

Shyer AE, Huycke TR, Lee C, Mahadevan L, Tabin CJ. 2015. Bending gradients: how the intestinal stem cell gets its home. *Cell* 161(3):569–80

Simsek MF, Chandel AS, Saparov D, Zinani OQH, Clason N, Özbudak EM. 2023. Periodic inhibition of Erk activity drives sequential somite segmentation. *Nature* 613(7942):153–59

Simsek MF, Özbudak EM. 2022. Patterning principles of morphogen gradients. *Open Biol.* 12(10):220224

Sokolowski TR, Erdmann T, ten Wolde PR. 2012. Mutual repression enhances the steepness and precision of gene expression boundaries. *PLOS Comput. Biol.* 8(8):e1002654

Sorre B, Warmflash A, Brivanlou AH, Siggia ED. 2014. Encoding of temporal signals by the TGF-β pathway and implications for embryonic patterning. *Dev. Cell* 30(3):334–42

Stanganello E, Hagemann AIH, Mattes B, Sinner C, Meyen D, et al. 2015. Filopodia-based Wnt transport during vertebrate tissue patterning. *Nat. Commun.* 6:5846

Stapornwongkul KS, de Gennes M, Cocconi L, Salbreux G, Vincent J-P. 2020. Patterning and growth control in vivo by an engineered GFP gradient. *Science* 370(6514):321–27

Stapornwongkul KS, Vincent J-P. 2021. Generation of extracellular morphogen gradients: the case for diffusion. *Nat. Rev. Genet.* 22(6):393–411

Steward R, Zusman SB, Huang LH, Schedl P. 1988. The *dorsal* protein is distributed in a gradient in early Drosophila embryos. *Cell* 55(3):487–95

Teimouri H, Kolomeisky AB. 2015. The role of source delocalization in the development of morphogen gradients. *Phys. Biol.* 12(2):026006

Tenzen T, Allen BL, Cole F, Kang J-S, Krauss RS, McMahon AP. 2006. The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice. *Dev. Cell* 10(5):647–56

The I, Bellaiche Y, Perrimon N. 1999. Hedgehog movement is regulated through *tout velu*-dependent synthesis of a heparan sulfate proteoglycan. *Mol. Cell* 4(4):633–39

Tkačik G, Gregor T. 2021. The many bits of positional information. *Development* 148(2):dev176065

Tsai TY-C, Sikora M, Xia P, Colak-Champollion T, Knaut H, et al. 2020. An adhesion code ensures robust pattern formation during tissue morphogenesis. *Science* 370(6512):113–16

Tuazon FB, Wang X, Andrade JL, Umulis D, Mullins MC. 2020. Proteolytic restriction of chordin range underlies BMP gradient formation. *Cell Rep.* 32(7):108039

Tukachinsky H, Kuzmickas RP, Jao CY, Liu J, Salic A. 2012. Dispatched and Scube mediate the efficient secretion of the cholesterol-modified hedgehog ligand. *Cell Rep.* 2(2):308–20

Umulis D, O'Connor MB, Blair SS. 2009. The extracellular regulation of bone morphogenetic protein signaling. *Development* 136(22):3715–28

Umulis DM, Othmer HG. 2013. Mechanisms of scaling in pattern formation. *Development* 140(24):4830–43

Umulis DM, Othmer HG. 2015. The role of mathematical models in understanding pattern formation in developmental biology. *Bull. Math. Biol.* 77(5):817–45

Verd B, Clark E, Wotton KR, Janssens H, Jiménez-Guri E, et al. 2018. A damped oscillator imposes temporal order on posterior gap gene expression in *Drosophila*. *PLOS Biol.* 16(2):e2003174

Vuilleumier R, Springhorn A, Patterson L, Koidl S, Hammerschmidt M, et al. 2010. Control of Dpp morphogen signalling by a secreted feedback regulator. *Nat. Cell Biol.* 12(6):611–17

Vyas N, Walvekar A, Tate D, Lakshmanan V, Bansal D, et al. 2014. Vertebrate Hedgehog is secreted on two types of extracellular vesicles with different signaling properties. *Sci. Rep.* 4:7357

Warmflash A, Zhang Q, Sorre B, Vonica A, Siggia ED, Brivanlou AH. 2012. Dynamics of TGF-β signaling reveal adaptive and pulsatile behaviors reflected in the nuclear localization of transcription factor Smad4. *PNAS* 109(28):E1947–56

Wartlick O, Jülicher F, Gonzalez-Gaitan M. 2014. Growth control by a moving morphogen gradient during *Drosophila* eye development. *Development* 141(9):1884–93

Wartlick O, Kicheva A, González-Gaitán M. 2009. Morphogen gradient formation. *Cold Spring Harb. Perspect. Biol.* 1(3):a001255

Wartlick O, Mumcu P, Kicheva A, Bittig T, Seum C, et al. 2011. Dynamics of Dpp signaling and proliferation control. *Science* 331(6021):1154–59

Wharton SJ, Basu SP, Ashe HL. 2004. Smad affinity can direct distinct readouts of the embryonic extracellular Dpp gradient in *Drosophila*. *Curr. Biol.* 14(17):1550–58

Wierbowski BM, Petrov K, Aravena L, Gu G, Xu Y, Salic A. 2020. Hedgehog pathway activation requires coreceptor-catalyzed, lipid-dependent relay of the Sonic hedgehog ligand. *Dev. Cell* 55(4):450–67.e8

Woods IG, Talbot WS. 2005. The *you* gene encodes an EGF-CUB protein essential for Hedgehog signaling in zebrafish. *PLOS Biol.* 3(3):e66

Wu H, Manu JR, Ma J. 2015. Temporal and spatial dynamics of scaling-specific features of a gene regulatory network in *Drosophila*. *Nat. Commun.* 6(1):10031

Xiong F, Tentner AR, Huang P, Gelas A, Mosaliganti KR, et al. 2013. Specified neural progenitors sort to form sharp domains after noisy Shh signaling. *Cell* 153(3):550–61

Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. 1991. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* 64(4):841–48

Yu D, Small S. 2008. Precise registration of gene expression boundaries by a repressive morphogen in *Drosophila*. *Curr. Biol.* 18(12):868–76

Zagorski M, Tabata Y, Brandenberg N, Lutolf MP, Tkačik G, et al. 2017. Decoding of position in the developing neural tube from antiparallel morphogen gradients. *Science* 356(6345):1379–83

Zecca M, Basler K, Struhl G. 1995. Sequential organizing activities of engrailed, hedgehog and decapentaplegic in the *Drosophila* wing. *Development* 121:2265–78

Zeng X, Goetz JA, Suber LM, Scott WJ, Schreiner CM, Robbins DJ. 2001. A freely diffusible form of Sonic hedgehog mediates long-range signalling. *Nature* 411(6838):716–20

Zhu J, Nakamura E, Nguyen M-T, Bao X, Akiyama H, Mackem S. 2008. Uncoupling Sonic hedgehog control of pattern and expansion of the developing limb bud. *Dev. Cell* 14(4):624–32

Zhu Y, Qiu Y, Chen W, Nie Q, Lander AD. 2020. Scaling a Dpp morphogen gradient through feedback control of receptors and co-receptors. *Dev. Cell* 53(6):724–39.e14

Zinski J, Bu Y, Wang X, Dou W, Umulis D, Mullins MC. 2017. Systems biology derived source-sink mechanism of BMP gradient formation. *eLife* 6:e22199

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