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Scaling of morphogen gradients

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Individuals of the same or closely related species can vary substantially in size. Still, the proportions within and between tissues are precisely kept. This adaptation of pattern with size termed scaling, is receiving a growing attention. We review experimental evidence for scaling, and describe theoretical models for mechanisms that scale morphogen gradients. We particularly note the Expansion–Repression mechanism, in which a diffusible molecule that positively regulates the morphogen gradient width is repressed by morphogen signaling. The Expansion–Repression circuit provides scaling in a robust manner and is readily implemented by a host of molecular mechanisms. We suggest means for identifying such a circuit in a system of interest.

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Introduction

The size of the embryo depends on genetic and environmental factors. It is therefore quite common for organisms of the same or closely related species to vary in size. Small and large embryos develop normally, and are successful in keeping accurate proportions within and between tissues. In other words, the body pattern of a developing animal is robust to size variations, demonstrating a capacity to dynamically adapt by scaling pattern with size.

Morphogen gradient is a widely used mechanism by which a developing tissue provides its cells with positional information [1]. In this paradigm, a localized group of cells secretes a molecule, the morphogen, to a larger field where it establishes a concentration gradient. Cells in the field respond to morphogen signaling in a concentration-dependent manner to induce a new pattern, so that cells close to the source, which sense high levels of morphogen, express one set of genes, while cells far from it, which sense a lower level, express other genes (Figure 1a). The ability to adapt to size variations requires scaling of the morphogen gradient with the size of the field. Simple analysis shows that merely increasing morphogen flux has a minor effect on the width of the gradient and cannot be used to maintain proportionate patterning (Figure 1b). Scaling within a large field entails a wider morphogen gradient, while a smaller field requires a proportionally narrower gradient (Figure 1c).

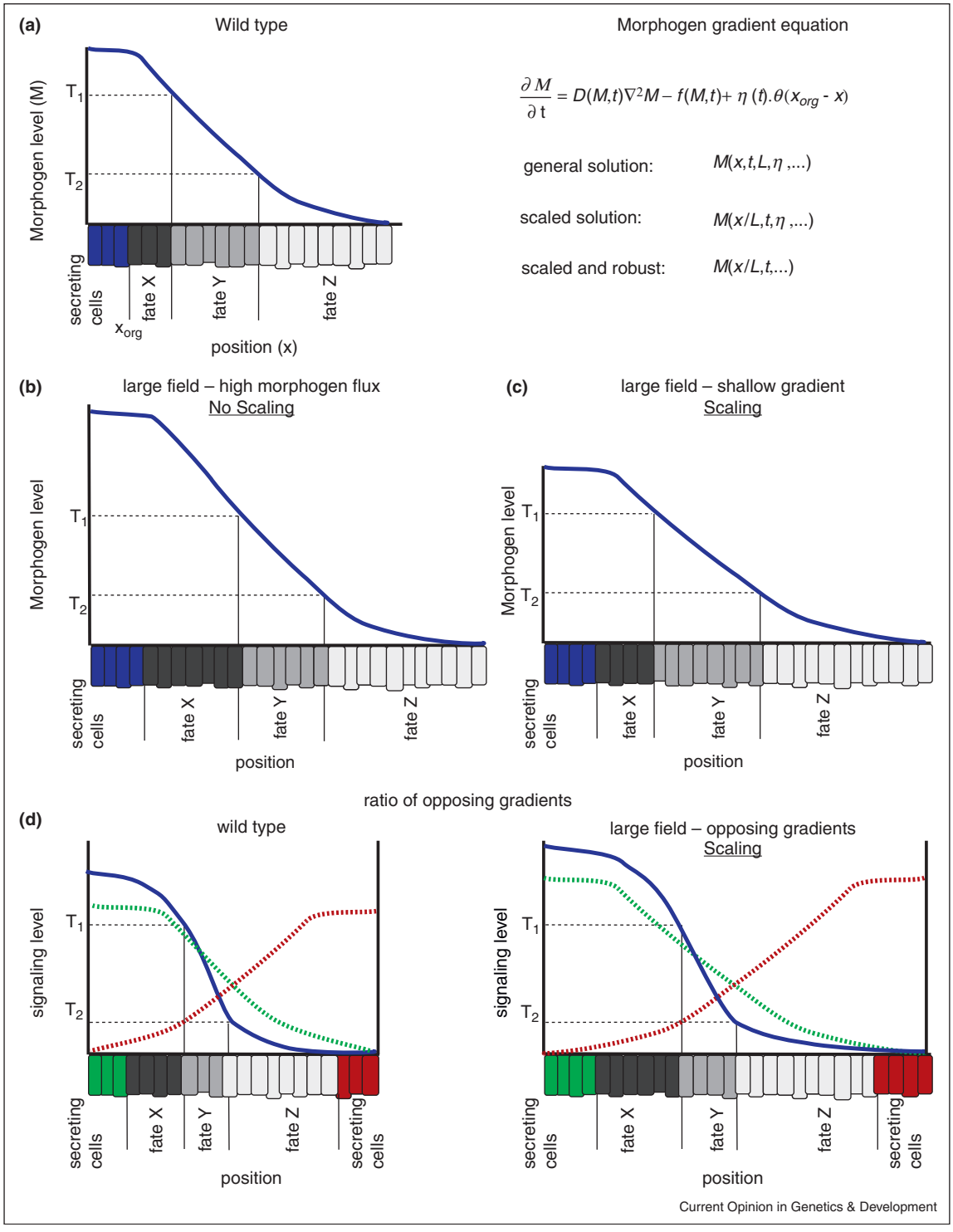
Experimental evidence for scaling

One of the earliest, and arguably the most dramatic, manifestation of scaling was noted by Hans Spemann and Hilde Mangold in their classical experiments on amphibian embryos [2,3]. In one experiment, an embryo was physically cut into dorsal and ventral halves. The ventral half failed to develop, while the dorsal half, containing what was later defined as the 'Spemann organizer', continued to develop into a small embryo of normal proportions. In a second experiment, the 'Spemann organizer' of a donor embryo was grafted onto the ventral side of a recipient embryo. The resulting chimera formed a secondary axis giving rise to 'Siamese twins'. Each twin was about half the size of a normal embryo but proportionately patterned.

Some 50 years later, Jonathan Cooke quantified scaling in this system by counting the number of cells composing the various tissues of *Xenopus laevis* embryos, comparing embryos of artificially reduced sizes, as well as embryos induced to duplicate their axes ('Siamese twins') [4]. Notably, in all embryos, the proportionate distribution of cells between tissues remained constant, despite large variations in the total number of cells and the differences in sizes. This striking finding led Cooke to question the morphogen gradient theory. Nevertheless, subsequent studies demonstrated that the *Xenopus* embryo is patterned by a morphogen gradient of Bone Morphogenic Proteins (BMPs) [5–7].

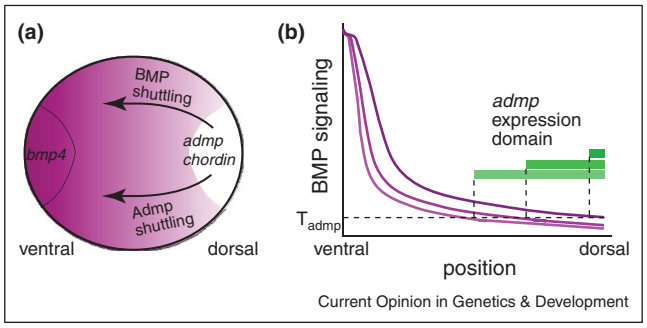
More recently, scaling was tested in systems where the gradient can be directly visualized. The *Drosophila* wing imaginal disc is one of the central paradigms for studying morphogen-based patterning. Dpp, a TGF-β ligand, is the primary morphogen patterning the anterior–posterior (AP) axis of the disc. To examine whether the Dpp activation gradient scales, disc size was manipulated by mutating the insulin signaling pathway, and the phosphorelation of Mad, one of the first steps in the Dpp signaling pathway, was monitored in the disc. Indeed, Dpp signaling gradient scaled with disc size, becoming sharper in smaller discs and wider in larger ones [8••].

Figure 1



Scaling of morphogen gradients. **(a)** Left panel: the morphogen gradient paradigm: the morphogen is secreted from a small number of cells (secreting cells, blue) and creates a gradient along a field. Cells close to the morphogen source sense high levels of the morphogen, above threshold T_1, and differentiate into fate X (dark gray). Cells that sense intermediate levels, between thresholds T_1 and T_2 adpot fate Y (medium gray). Cells far from the source sense low levels, below threshold T_2 and differentiate into fate Z (light gray). Right panel: general equation describing a morphogen gradient. M is the morphogen concentration, $D(M,t)$ and $f(M,t)$ its diffusion and degradation terms, which may depend on the morphogen concentration through feedbacks and on time. $\eta(t)$ is the morphogen production term, typically restricted to a small group of cells at the edge of the field $\{x|0 < x < x_{org}\}$. The solution for this equation, $M(x,t,L,D,f,\eta)$, will scale with size if it is a function of x/L, the relative position rather than of x, with L the size of the field.

Figure 2



Scaling of the BMP gradient in *Xenopus laevis* embryos. **(a)** Schematic representation of the BMP gradient (magenta) along the dorso-ventral axis of *Xenopus laevis* embryos. *admp*, a BMP ligand, is repressed by BMP signaling and therefore its expression is confined to the dorsal most region of the embryo, the Spemann organizer (white) where it is co-expressed with BMP inhibitors such as Chordin. *bmp4*, another BMP ligand is expressed at the ventral pole, where BMP signaling is highest. Admp, as well as the other BMP ligands are shuttled ventrally by their inhibitor Chordin. **(b)** Shuttling of Admp and its accumulation along the DV axis leads to the expansion of the BMP gradient (magenta) and the repression of *admp* expression (green bars) in virtually the entire field. Light shades of magenta denote earlier stages of the dynamics, dark shades denote later stages. Close to steady state, *admp* repression fixes signaling at the dorsal region of the field to T_{admp}, the *admp* repression threshold.

More recently, a study from the Gonzalez-Gaitan lab extended these findings to the growing disc by following the Dpp gradient itself and its downstream targets during the final stages of larval growth, where the disc grows by twofold [9••]. During most of this period, the Dpp activation gradient scaled with disc size. This study hinted at the mechanistic explanation for scaling, showing that Dpp degradation rate decreases with increasing disc size.

The first morphogen gradient to be visualized was Bicoid [10,11]. Bicoid is the transcription factor patterning early dipteran embryos including *Drosophila*. The gradient of Bicoid and the spatial domains of its downstream genes were quantified in embryos from different dipteran species varying by fivefold in size [12,13]. In all species examined, the Bicoid gradient and the expression domains of its downstream genes scaled with the size of embryo. Notably, the ability to scale the gradient was not due to differences in the sequence of the Bicoid protein itself, but to factors in the embryonic cytoplasmatic environment.

Mathematical analysis of scaling mechanisms

Models of morphogen gradients usually assume a morphogen that is secreted locally and diffuses along the field (Figure 1a). A main property relevant to our discussion is that the only way to obtain scaling in this simple model is by assuming that first, morphogen does not degrade across the field; second, there is a 'sink' of morphogen at the edge of the field and third, morphogen level at the source is kept constant, independent of tissue size. These constraints are rarely implemented, in particular because morphogen loss is inevitable during its diffusion or endocytosis following signaling [14,15••]. Clearly, in the general case, scaling requires specialized mechanisms.

Theoretical studies pointed at molecular circuits that could scale morphogen gradients. One group of mechanisms involves two morphogen gradients, emanating from opposing edges of the field. It was shown that the ratio of these morphogens scales with the size of the field (Figure 1d) [16,17•,18]. This mechanism was proposed, for example, in the context of the Bicoid gradient, but could apply more globally. Indeed, few systems were described in which two opposing gradients affect cellular fates [19,20]. A limitation of this mechanism is that it is sensitive to small deviations in the parameters defining the two opposing gradients. Furthermore, in most cases, scaling is achieved for a specific region only (e.g. the middle of the field) but not at other positions, leading to partial scaling [15••,21,22]. An alternative annihilation model may provide scaling, provided some specific biochemical interactions [17•].

Another group of mechanisms is based on geometric considerations. For example, it was suggested that Bicoid is degraded only inside the nuclei of the *Drosophila* embryo. As the number of nuclei is independent of embryo size, degradation becomes effectively lower in larger embryos, which could provide scaling [23]. A similar mechanism was invoked to explain the scaling of the Dpp gradient in the wing imaginal disc [24•]. Recently, it was suggested that the apico-basal growth in the wing imaginal disc may account for scaling in this system [25]. Other theoretical mechanisms suggest that the ubiquitous production of a regulatory element whose concentration is affected by size can lead to scaling [26•,27]. These mechanisms may apply to specific cases, but cannot explain scaling in most systems, where degradation and diffusion of the morphogen are also regulated by morphogen signalling.

The BMP activation gradient in the early *Xenopus* embryo presents an interesting case study. Work from the De Robertis lab identified Admp, a BMP ligand, as a key factor for scaling, because depletion of this protein

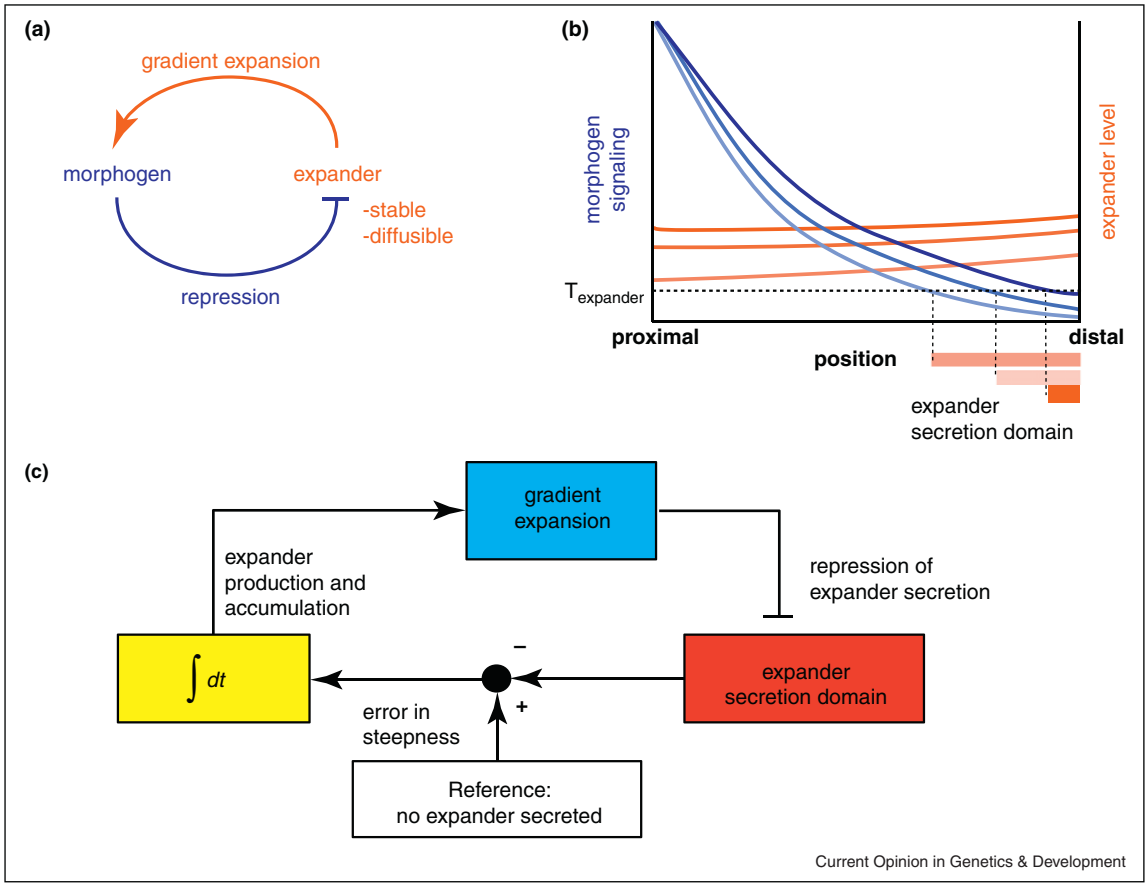
(Figure 1 Legend Continued) Robustness to the morphogen production terms implies that the solution is independent of the value of η. **(b)** Modulation of morphogen flux does not account for scaling. Increasing morphogen flux in larger fields changes the proportions between different cell fates in the field relative to the wild type size. **(c)** Scaling of morphogen gradients requires modulation of the sharpness of the gradient to maintain the same proportions of cell fates. **(d)** The ratio (blue) between two gradients emanating from opposing edges of the field (green and red) can provide scaling of a signaling gradient. Left panel: wild type field; right panel: larger field.

prevents the proper development of dorsal-half embryos [28••,29]. *admp* is repressed by BMP signaling, whereas the BMP ligand, *bmp4*, is activated by BMP signaling [7]. This leads to the seemingly paradoxical situation where *admp* expression is confined to the dorsal region, where BMP signaling is lowest and the prominent BMP inhibitor *chordin* is expressed, while its activity is required at the ventral region (Figure 2a). By mathematically analyzing this system, we showed that this design can scale the BMP activity gradient [30••]. We found that activity of several BMP ligands (Admp and Bmp2/4/7) allows flexibility in gradient width. High levels of Admp relative to Bmp2/4/7 leads to wide gradients, whereas low Admp levels result in narrow gradients. Accumulation of Admp, therefore, expands the gradient in time. Critically, Admp stops accumulating only when the gradient is wide enough to suppress *admp* expression in the entire field (Figure 2b). This provides effective means to measure embryo size, and scale the gradient accordingly.

The Expansion–Repression mechanism for scaling

The basic scaling mechanism described above can be generalized to a class of simple molecular circuits, which we denote as 'Expansion–Repression' [15••]. The Expansion–Repression mechanism assumes a single morphogen and an additional diffusible molecule, the expander. The latter expands the morphogen gradient, either directly or indirectly (e.g. by facilitating diffusion or inhibiting its degradation), while morphogen signaling represses expander production or secretion. Both molecules are present in the extracellular milieu, and function in a non-cell autonomous manner (Figure 3a).

Figure 3



The Expansion–Repression mechanism. **(a)** The Expansion–Repression feedback topology. The morphogen (blue) represses the secretion of an expander (orange). The expander, which is diffusible and stable, expands the morphogen gradient. **(b)** Expansion of the morphogen gradient (pale to bright blue) leads to the gradual restriction of the expander secretion domain towards the distal region of the morphogenic field (pale to bright orange bars). $T_{expander}$ is the threshold for expander secretion repression. The gradient continues to expand until the expander is repressed in almost the entire field. The expander accumulates during the expansion of the gradient (pale to bright orange), such that larger fields will require higher levels of the expander. **(c)** An integral feedback lies at the heart of the Expansion–Repression mechanism. Expander secretion (red) is compared to the reference, no secretion of the expander (white). The error, which is the expander-secretion domain, translates into expander secretion and accumulation over time (yellow). Increase in expander levels leads to gradient expansion (blue), which results in restriction of the expander-secretion domain. The control circuit stabilizes when the error is zero, that is, when the expander is not secreted.

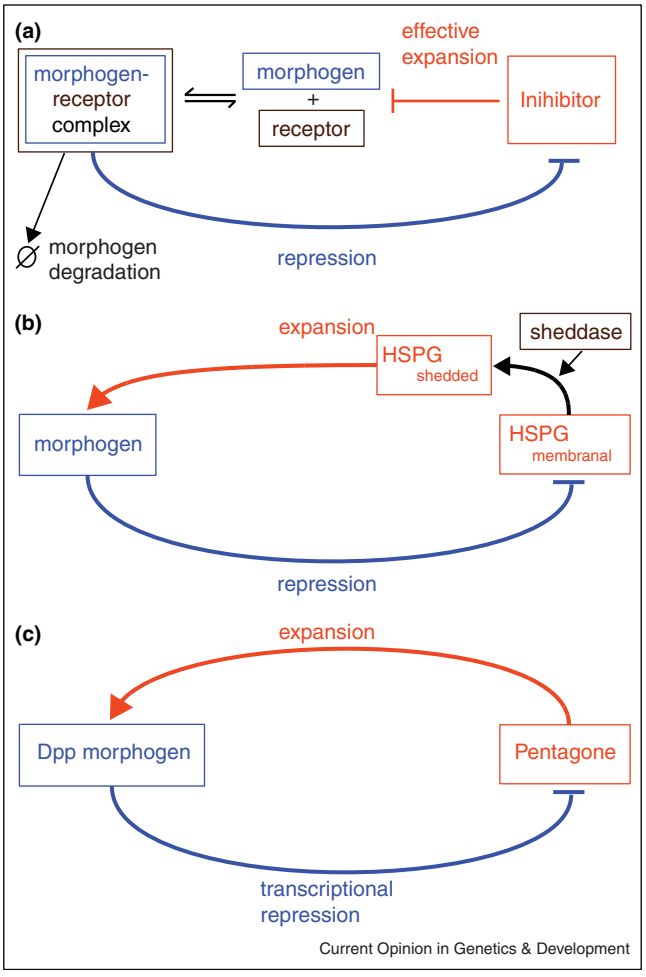
Scaling is achieved naturally: the morphogen gradient is initially sharp, enabling the accumulation of the expander. As a result, the gradient expands until its level at the edge of the field is sufficient to inhibit further secretion of the expander. The signaling level at the distal-most part of the field is therefore fixed at the level repressing expander secretion (Figure 3b). The expander level at steady state couples the size of the field and the slope of the gradient, with larger fields containing more expander molecules.

Identifying molecular circuits potentially implementing the Expansion–Repression mechanism

The simple design of the Expansion–Repression mechanism and the wide range of molecular interactions that can be used to generate it, make such a circuit potentially applicable to many biological systems. An important lesson from the *Xenopus* BMP patterning system is that the expander may be difficult to identify, because it can have other biological functions. For example, Admp is not only an expander, but also a BMP ligand. In other systems, different elements such as inhibitors can also be expanders, given that they are diffusible and are repressed by morphogen signaling. Consider the common situation where a morphogen is degraded following interaction with its receptor. Binding of the inhibitor to the morphogen prevents not only morphogen signaling but also morphogen degradation, thereby expanding the morphogen gradient. In this case, the inhibitor is also an expander (Figure 4a) [15••,31•]. One can think of other implementations of such scaling mechanisms by the regulation of proteoglycan shedding (Figure 4b).

Several examples come to mind. First, *Drosophila* Pentagone was recently characterized as a modulator of the Dpp gradient in the *Drosophila* wing imaginal disc [32]. Pentagone is secreted, repressed by Dpp signaling and expands the Dpp gradient. We have recently shown that it is indeed required for scaling of the gradient and functions as an expander in this system [33•] (Figure 4c). Also in the wing-disc, expression of the proteoglycan *dally-like* is repressed by Wingless signaling, and its shedding is mediated by the hydrolase Notum [34–37]. Shed proteoglycans may function as expanders, provided that they expand the morphogen gradients repressing their expression. Similarly, if a fraction of the GPI-linked Gas1 protein is shed, it can function as an expander for the Shh gradient in the developing vertebrate neural tube [38,39]. Other possible expanders include the secreted Wnt inhibitors Frzb and Crescent, which were shown to enhance Wnt diffusion in the *Xenopus* embryo [31•]. Having the Expansion–Repression paradigm in mind can, therefore, guide research towards identifying specific scaling mechanisms.

Figure 4



Possible implementations of the Expansion–Repression mechanism.
(a) A morphogen inhibitor can function as an expander: when degradation of the morphogen is mediated by interaction with its receptor, inhibition of the morphogen binding to the receptor decreases the morphogen degradation rate. Therefore, the inhibitor is effectively an expander.
(b) Shedding of HSPGs can be a part of an Expansion–Repression mechanism. In the case where shed HSPG expands the gradient, repression of HSPG expression in the presence of a sheddase (HSPG shedding protein), will lead to scaling through Expansion–Repression.
(c) Pentagone is an expander for the Dpp gradient in the AP axis of the *Drosophila* wing imaginal disc. *pentagone* is transcriptionally repressed by Dpp signaling, and expands the Dpp gradient through interaction with Dally, a *Drosophila* proteoglycan.

Prospects

One of the difficulties in identifying scaling mechanisms is to experimentally differentiate between proteins contributing to scaling versus proteins that impinge on patterning *per se*. In fact, the same protein may have a dual role in these processes, as scaling is likely to be an integral part of the patterning mechanism. Theoretical models are therefore invaluable in classifying mechanisms that can provide scaling, guiding the quest for their

implementation in the different systems. Further testing for such mechanisms requires the ability to measure patterning at high precision, in parallel to manipulating the size of the field.

Size can be modulated in different ways: artificially (e.g. mutations), during growth and between species. An open question is whether the same mechanism functions in all these situations, namely whether the same mechanism that scales the pattern during growth also enables scaling the pattern between species and maintains proportionate patterning upon genetic manipulations. Deeper understanding could provide insights into the scaling during evolution.

Our discussion here focused on models which treat the tissue as a static field. This, however, is not always the case. Morphogens affect patterning, and in many cases can induce the proliferation and movement of cells. These effects can contribute to scaling the system by co-ordinating growth, morphogenesis and patterning [8••,9••,40,41]. Theoretical and experimental analysis of such a coupling is more difficult, but holds promise for identifying new scaling mechanisms, and better understanding of the basic developmental processes co-ordinating pattern with size.

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