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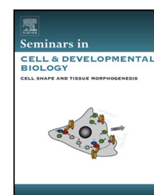

Review

Dpp/BMP signaling in flies: From molecules to biology

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ABSTRACT

Decapentaplegic (Dpp), the fly homolog of the secreted mammalian BMP2/4 signaling molecules, is involved in almost all aspects of fly development. Dpp has critical functions at all developmental stages, from patterning of the eggshell to the determination of adult intestinal stem cell identity. Here, we focus on recent findings regarding the transcriptional regulatory logic of the pathway, on a new feedback regulator, Pentagone, and on Dpp's roles in scaling and growth of the *Drosophila* wing.

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1. Introduction

The *decapentaplegic* (*dpp*) gene was first described in 1982 by Gelbart and colleagues as a gene complex; mutations in *dpp* produced multiple phenotypes by affecting one or several of the 15 imaginal discs of the *Drosophila* larvae [1]. Only a few years later, sequencing of the *dpp* locus unraveled a transcript predicting *dpp* to encode a member of the TGF-β family of signaling molecules [2]. Since then, roughly 1600 papers were published on the *dpp* locus and/or the function of the Dpp protein. As it turned out, Dpp is involved in numerous processes throughout all developmental stages, from stem cell maintenance to regeneration. In many instances, research on *Drosophila dpp* has provided important insights into related processes in vertebrates.

It is certainly for its numerous important biological roles that *dpp* has been so widely studied in flies. In the past few years, the role of Dpp, which was mostly studied in the control of patterning of embryos and imaginal discs [3–10], has extended to other developmental stages and to more novel, emerging themes in developmental biology. Dpp signaling has been associated with stem cell function and regulation. Some time ago already, Dpp was found to be instrumental in maintaining self-renewal of germ line stem cells in the stem cell niche [11] (reviewed in Refs. [12,13]). More recently, Dpp has been implicated in size control of the hematopoietic niche [14], and in the control of the number of stem cells in the adult midgut of *Drosophila* [15]. Furthermore, Dpp determines regional stem cell identity in the regenerating adult *Drosophila* gastrointestinal tract [16]. These studies emphasize that Dpp signaling is important not only in tissue patterning, but also plays an important role in tissue homeostasis.

Dpp controls various cellular activities, from cell division to cell migration. It comes somewhat as a surprise, then, that in all cases that have been studied in detail thus far, the effects of the loss-of-function phenotype observed in flies is due to, or can be attributed to a large extend to, the regulation of transcription of downstream factors. This might be due to the approaches taken in *Drosophila* to study gene (protein) function, which is tightly linked to genetics and less often involves more protein-based methods such as mass spectrometry. In Section 2, we outline the state of the art regarding the transcriptional regulation in response to Dpp signaling. Special emphasis is given to the recently described feedback regulators.

Dpp has gained broader interest in the scientific community as it represents the first bona-fide secreted morphogen [17,18]. One of the most controversial aspects of Dpp signaling is the formation of the Dpp gradient during wing development, which we will not discuss in this review, but would like to direct the interested readers to a recent excellent overview of the subject [19]. Sections 3 and 4 summarize what is known about Dpp acting as a morphogen in the wing imaginal disc, its role in scaling and in growth control. Since research in this field is ongoing and different approaches are being pursued, no consensus has emerged yet as to the role of Dpp in these intriguing biological processes, and we propose that the way forward is to use more quantitative approaches to resolve these issues.

2. Molecular players, transcriptional control and feedback regulation

Most or all of the Dpp functions in *Drosophila* development were assigned to the capacity of the signaling pathway to control transcription of target genes. For this reason, we will outline and discuss in quite some detail the signaling pathway, its cytoplasmic and nuclear components, and its regulatory logic.

The core Dpp signaling pathway includes only few components and its structure is relatively simple [20,21]. Signaling starts when Dpp dimers (or, in some instances, heterodimers with one of the other two *Drosophila* BMPs, Screw and Glass bottom boat) assemble receptor complexes at the plasma membrane. Identical to vertebrate BMP signaling, receptors comprise type I and type II subunits. Most effects of Dpp are mediated by the type I receptor Thickveins (Tkv) which becomes phosphorylated and activated by the type II receptor Punt upon ligand binding. Activated Tkv in turn phosphorylates the *Drosophila* Smad Mothers-against-Dpp (Mad; P-Mad in its phosphorylated form), which associates with the co-Smad Medea and accumulates in the nucleus (Fig. 1b). P-Mad/Med complexes bind to GC-rich motifs in control regions of numerous genes and, in concert with additional transcription factors, regulate their transcription.

Nuclear responses to Dpp have been mostly analyzed in two contexts, the establishment of the dorso-ventral axis during early embryonic development and the larval development of the wing. In both cases, Dpp establishes an activity gradient that regulates expression of target genes in a concentration-dependent manner. Although we are still missing a thorough, genome-wide analysis of

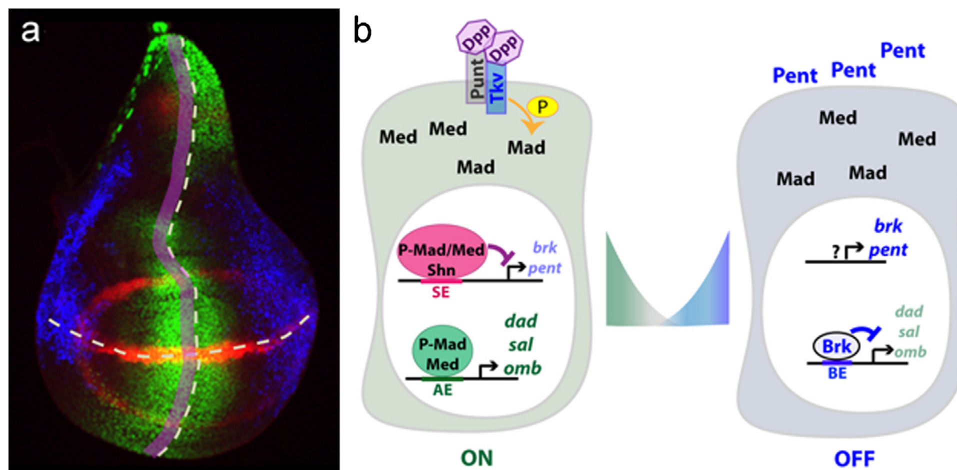

Fig. 1. The activity profile and the transcriptional logic of the Dpp/BMP pathway. (a) Wing disc with reporters for Dpp activity gradient (*dad-GFP* in green), *brk* expression (blue, brk-Gal4 > UAS-mCherry), and *wg* expression (red, *wg-lacZ*). A/P and D/V boundaries are marked with dotted lines. Dpp expression domain is marked with a purple stripe. (b) Transcriptional activity in cells with two extreme levels of Dpp signaling levels are schematized; a medial cell in green and a lateral cell in blue. The question mark highlights the unknown transcriptional activator(s) of *brk* and *pent*. SE: Silencer Element, AE: Activating Element, BE: Brinker Element.

Dpp response, studies on individual target genes have revealed key molecular insights in Dpp-dependent transcriptional regulation. In the following we focus on two important and interconnected features of *Drosophila* Dpp signaling, namely the ability to directly repress gene transcription and the role of the transcription factor Brinker (Brk) in Smad-dependent gene regulation.

2.1. Transcriptional repression: Brinker and the Silencer Elements

Brk is a sequence-specific transcriptional repressor that contains an N-terminal homeobox-like DNA-binding domain [22–24]. The larger, C-terminal part of the protein bears interaction motifs for the recruitment of multiple co-repressors, including CtBP (C-terminal Binding Protein) and Groucho [25–27]. Importantly, Brk antagonizes Dpp-responses in numerous, if not all, processes (reviewed in Refs. [3,28]). During early embryogenesis, for example, most genes that are activated by the steep dorsal-high to ventral-low P-Mad/Med gradient in the dorsal ectoderm are simultaneously repressed ventrally by Brk. During this process, Brk is produced under the control of the Dorsal morphogen gradient in the ventral neurogenic ectoderm, in a region that abuts the dorsal ectoderm. Although we lack direct evidence, genetic studies suggest that Brk distributes in a gradient that is inverse to the Smad gradient [29,30]. While a few high Dpp threshold genes, such as *race*, do not require Brk to establish their expression boundaries, other genes integrate information from both Brk and P-Mad/Med [29,31–36]. Such genes are activated by P-Mad/Med complexes and simultaneously repressed by Brk, which establishes their ventral expression limit. Notably, and similar to what has been shown for other morphogen gradients (such as Bicoid, for example), responses to the Dpp/Brk gradients are anything but linear, as *cis*-regulatory regions of Dpp targets integrate additional inputs for proper expression, including substantial cross-regulation between target genes [33,37,38].

The contribution of the Brk repressor activity to the nuclear responses to Dpp is even more pronounced during larval wing development. First, all genes identified so far to be positively regulated by the Dpp gradient in the wing imaginal disc are subject to Brk regulation and, secondly, Dpp signaling directly represses *brk* transcription in this tissue [22–24,26,39–48]. Thus, the medial to lateral Dpp activity gradient in the developing wing imaginal disc generates an inverse gradient of *brk* transcription and, consequently, Brk protein distribution (Fig. 1). Dpp-targets in the wing, such as *vestigial* (*vg*), *optomoter blind* (*omb*) and *spalt* (*sal*), receive dual Dpp input: Smad complexes provide activating cues while Brk antagonizes activation in lateral regions of the imaginal disc. Since Dpp targets display differential sensitivity to Brk levels, their expression is activated in nested domains centered on the source of the ligand [41,48].

Dpp-induced repression of *brk* transcription was found to be direct and dependent on short sequences in the regulatory regions of *brk*, termed *Silencer Elements* (*SEs*), and Schnurri (Shn), a large Zn-finger protein that acts as a transcriptional repressor in the regulation of *brk* [40,41,43,46,49]. The *SE* is a GC-rich sequence of the consensus GRCGNC$(N)_5$GTCTG (R: purine, N: any nucleotide) and comprises Smad binding motifs separated by a linker sequence. Dpp-activated Smad complexes dock to the SEs as trimers; the MH1 domains of the two P-Mad molecules of a Smad trimer bind to the GRCGNC motif, while Med binds to the GTCTG motif. Once the trimer assembles on the *SE*, it can recruit Shn. Shn imposes two very specific sequence constrains to the element: a spacer of 5 nucleotides between the two Smad binding sites and a T at nucleotide position 4 in the Med binding site (GTC$\underline{T}$G) [46]. If any of these requirements is not fulfilled, the Smad complex can still bind to the element but Shn recruitment to the complex and transcriptional repression are abolished. This indicates that the specific arrangement and sequence of Smad binding sites found in the *SE* accommodate Shn binding and transcriptional repression, and that other constellations might be present to achieve different outcome and/or provide platforms for the recruitment of different Smad-cofactors.

2.2. Transcriptional activation: the Activating Elements

Evidence for the presence of other constellations came with the analysis of the transcriptional regulation of *daughters-against-dpp* (*dad*), which codes for the only known inhibitory Smad (iSmad) in *Drosophila* [50,51]. Transcription of *dad* is positively regulated by Dpp in all contexts tested and *dad* expression is very often used as a marker for active Dpp signaling. As most Dpp-targets, *dad* receives activating and repressive input by Smads and Brk, respectively. Both cues integrate on short bipartite elements, which, in analogy to the *SE*, are referred to as *Activating Elements* (*AEs*) (Fig. 1b). The *AE* is similar in structure and sequence to the *SE*, but deviates from the later in key nucleotide positions that reverse its activity. The motif, as derived from the *dad* analysis, *GGCGYC*$(N)_5$*GTCV* (V: any nucleotide except T), still allows for Smad trimer binding but excludes Shn recruitment because of the modification in the last nucleotide position. Instead, the Mad binding site present in the *AE* (*GGCGYC*) is also a Brk binding motif (consensus for Brk binding: *GGCGYY*) and it has been shown that Brk and Smads compete for binding the *AE* [50]. How much of the antagonism between Brk and Smad in Dpp-dependent gene regulation can be attributed to binding to overlapping sites is not yet clear. It is also unclear whether transcriptional activation by the *AE* requires co-activators to be recruited to the *AE*/Smad complex. Such factors, in analogy to the events on the *SE*, might impose additional sequence constraints on the *AE*. Alternatively, the inherent trans-activator properties of Smad proteins might be enough for transcriptional activation with no additional proteins involved. Consistent with this later hypothesis, increasing the linker length of the *AE* results in variants that are still able to bind Smad complexes and activate transcription in cell culture reporter assays [52]. Although an *in vivo* validation of such experiments is still pending, it might be that the *AEs* are not as stringent in their sequence requirements as the *SE*. For example, some of the GC-rich sequences that have been identified in Dpp-dependent enhancers and demonstrated to bind purified Smad proteins, might correspond to *AE*-variants that assemble Smad-trimers in a signal dependent manner. In this scenario, the *SE*, and possibly other yet uncharacterized motifs, might represent specialized variants of *AEs* that have evolved to recruit specific Smad co-factors that impinge on the elements' regulatory properties and functions.

Despite a number of open questions, both the *SE* and the *AE* motifs have provided valuable insights into Dpp signaling as they often provided direct molecular links between Dpp function and target gene regulation and even allowed for *de novo* identification of effectors and regulators of the signaling pathway. Indeed, a number of genes and enhancers have been shown to contain such motifs and in many cases the functionality of such elements has been directly verified. For example, Shn- and *SE*-dependent repression seems to be active during early *Drosophila* embryonic development and to contribute to shaping the dorsal expression border of neurogenic genes [53,54]. *SE* elements are also found in the loci of antimicrobial peptide genes and have been suggested to mediate Dpp's function in attenuating immune responses after wounding and infection [55]. In addition, *SEs* have been successfully used in motif-based searches to identify novel proteins which turned out to be key effectors or regulators of the signaling pathway – an example of such a protein is given below [46,56]. Similarly, phylogenetically conserved *AEs* have been identified in genomic loci of several

key developmental genes and mediate their Dpp-responsiveness [50,57].

2.3. A pathway of many feedbacks

As with other signaling pathways, Dpp-signaling is subject to extensive feedback regulation [7]. Many of the core components of the pathway and additional tissue- and context-specific accessory regulators of Dpp signaling are themselves regulated by the pathway. This regulation happens mostly, if not exclusively, at the transcriptional level. Feedback regulation might affect different levels of the signal transduction cascade. For example, the iSmad Dad is directly activated by Dpp signaling, and similar to its vertebrate homologs, downregulates signaling by blocking access of Mad to the activated receptor complex [51]. This negative regulatory feedback loop has been suggested to buffer against perturbation of receptor activity and to confer robustness to the activity gradient in the wing disc [58]. The most impressive examples, however, both in terms of number and diversity of the molecular nature of the regulators, come from feedback regulators that act outside the cell or at the cell membrane. Such regulatory mechanisms affect the distribution, stability and activity of the ligands and receptors and have been predominantly studied in Dpp morphogen gradient formation in the early embryo and the wing imaginal disc.

In the blastoderm embryo, the steep BMP activity gradient is generated by the transport of Dpp-Scw heterodimers to the embryo's dorsal midline. Dorsal accumulation of the ligand dimer is an evolutionarily conserved process that depends on a shuttling complex consisting of Short Gastrulation (Sog) and Twisted Gastrulation (Tsg), on the protease Tolloid (Tld), which cleaves the complex to release ligands, and on Collagen IV, which both catalyzes the assembly of the ligand shuttling complex and immobilizes Dpp [5,59–62]. Proper formation of the gradient has recently been shown to depend on feedback regulation provided by Eiger (Egr, the *Drosophila* tumor necrosis factor α) and Crossveinless-2 (Cv-2, a BMP-binding membrane protein), which promote and antagonize BMP signaling, respectively [61,63]. The transcription of both genes depends on the Dpp-target Zen; however, direct activation by Smad complexes is also likely to occur, since, *AEs* are present in an early embryonic enhancer of *cv-2* and Zen has been shown to cooperate with Smads in the activation of several genes in the same context [31,33,50,64].

In the wing imaginal disc, regulatory feedback is crucial and involves receptors, co-receptors and extracellular proteins which, in concert, control the distribution of the two BMP ligands, Dpp and Gbb, and shape the activity gradient. Receptors are obviously crucial for local signaling, and, at the same time, influence long-range ligand distribution as they can trap ligands and channel them into endocytosis. The importance of receptor levels on the gradient profile is reflected in the pattern of Tkv distribution across the morphogen field. Tkv levels are lowest at the source of the ligand and highest in lateral cells and this profile is shaped by transcriptional repression by both Hh and BMP signaling [65,66]. This distribution is crucial for the shape of the BMP activity gradient: modest levels of Tkv near the production source allow for ligands to move laterally, thus contributing to the establishment of the long-range activity gradient. Indeed, tissue-specific impacts on the regulation of receptor levels seems to be one of the mechanisms by which the range of BMP signaling is altered in a variety of contexts, including the ovarian stem cell niche and the haltere imaginal discs [67–70]. Similarly, Dally, a GPI-anchored heparan sulfate proteoglycan of the glypican family, has an elaborate pattern of expression along the anterior–posterior axis, which is shaped by multiple signaling cues, including BMPs. Dally binds and stabilizes Dpp at the cell surface and has been shown to be involved both in signaling (acting as a co-receptor) and in ligand dispersion, probably by handing-off Dpp from one cell to the other [71–77]. Altogether, the availability and the stoichiometry of Tkv and Dally at a given position of the morphogen field determine both the local signaling activity as well as the amount of ligands that become available for long-range signaling. In addition to their regulation at the transcriptional level, the dynamics of the receptors and co-receptor can be also modified by interactions with extracellular regulators. One recent example is the secreted protein Pentagone (Pent) which is essential for proper formation of the Dpp activity gradient in the wing disc and other tissues [56,78,79]. In the absence of Pent, P-Mad levels are abnormally high near the source of the ligand and the gradient is shorter and steeper. The increase of short range signaling at the expense of long-range signaling in *pent* mutants stems from imbalances in the receptor/co-receptor system. Pent is a secreted protein that directly interacts with Dally at the cell surface and is required for the activity of the glypican in long-range gradient formation but not for its co-receptor activity [56,78]. Thus, in the absence of Pent, Dpp is "consumed" locally and is lost for long range signaling. Importantly, while Pent seems to regulate signaling in medial cells, the production of *pent* is confined to lateral cells of the disc through direct, *SE*- and Shn-dependent transcriptional repression. Consequently, secreted Pent forms an extracellular gradient that is inverse to (and depends on) the Dpp gradient (Fig. 1) [56]. It was proposed that this feedback circuit provides the Dpp gradient with the capacity to directly regulate the amount of Pent that reaches medial cells and thus to control and correct the gradient shape according to its demands [80,81]. We further discuss this potential role of Pent in Section 4.

3. Dpp and growth control

The requirement for Dpp for the growth of the wing is undisputed. Mutant flies that lack Dpp expression in the developing wing imaginal discs fail to form wings, and patches of cells that cannot transduce Dpp are eliminated from the wing blade [82]. In contrast, patches of wing imaginal cells expressing ectopic Dpp, induce proliferation in surrounding cells, sometimes leading to striking organ duplications [83]. Hence, ectopic Dpp can induce proliferation of an extra tissue and, at the same time, patterns it the same way as the wild type tissue. These striking phenotypes, combined with the central expression pattern of Dpp along the anterior–posterior compartment boundary, have inspired much work on this model system, resulting in many models of wing growth, in which Dpp plays a pivotal role for growth and for patterning. These models can be grouped into two classes, being either instructive or permissive (also reviewed in Refs. [84,85]).

3.1. Models in which the Dpp gradient drives proliferation (instructive)

Given that Dpp is a potent growth factor, it is expected that growth takes place where Dpp activity is highest. In reality, the pattern of proliferation is roughly uniform across the wing disc while Dpp activity is highly graded (Figs. 1a and 2a) [86,87]. The very first model that attempted to explain this paradoxical situation came from Day and Lawrence who suggested that cells respond to the steepness of the Dpp gradient rather than to its absolute amounts [88]. Henceforth, this model will be referred to as *the slope model*. In *the slope model*, the gradients are steep in small discs and cells continue to grow and divide. As the tissue grows, the morphogen gradient flattens. When the concentration difference sensed across a cell falls below a certain threshold, the cells stop dividing. This model can explain why cell division occurs at similar rates all across

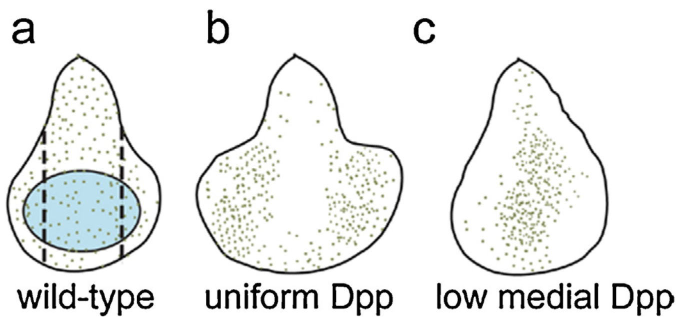

Fig. 2. The enigmatic relationship between the Dpp signaling and proliferation control. Schematics of BrdU patterns in late stage wing discs of indicated genotypes. The pouch, which will form the adult wing, is highlighted in blue and medial *versus* lateral disc regions are separated by dotted lines in (a).

the disc, since the slope of a morphogen gradient would be read locally at every point in a field of cells [88].

The slope model predicts that a flat Dpp concentration gradient would have no effect on the growth, but this prediction is not met and ubiquitous expression of Dpp in the disc induces significant growth [89,90]. This finding dampened the popularity of *the slope model*, until Rogulja and Irvine demonstrated that juxtaposing cells with largely different levels of Dpp pathway activity stimulates cell proliferation [87]. This stimulation was achieved either by elevating or by lowering the level of pathway activity relative to that in neighboring cells, suggesting that cells have a way of comparing their relative Dpp signaling levels and correct for any irregularities in the gradient by proliferating. Rogulja and Irvine also observed that uniform pathway activity actually inhibits cell proliferation in the medial disc, as evidenced by lower levels of BrdU incorporation in medial cells (Fig. 2b). This is in accordance with *the slope model* since a steep gradient of Dpp is a prerequisite for proliferation in this model. However, and as was previously observed, uniform pathway activity induces growth, especially in the lateral parts of the disc (Fig. 2b). These observations, along with the previous findings that *tkv* mutant cells are eliminated from the wing proper but can be recovered in lateral parts of the disc [82], indicated that Dpp might regulate growth differently in different parts of the disc. It was postulated that the slope of the gradient drives proliferation in the medial disc during development, while in the lateral disc, proliferation depends on absolute levels of Dpp [87]. These observations raised the question of how medial *versus* lateral identity is determined, or why cells respond differently depending on their location.

A different model that attempts to explain uniform growth under direct control of graded Dpp activity proposes that the cells monitor the temporal changes in Dpp signaling levels instead of the slope of the Dpp gradient to determine when to divide [86]. The model is based on careful examination of a Dpp-GFP fusion protein that was expressed in the *dpp* stripe during larval wing development using the UAS-Gal4 system. González-Gaitán and collaborators found that while the shape of the Dpp-GFP gradient in the posterior compartment did not change, its levels constantly increased during development. As a result, and given that long-range cell movements are rare in the wing epithelium, all cells experience the same relative increase in Dpp-GFP levels. Correlation of this constant increase in Dpp-GFP levels with cell division frequencies led to the hypothesis that cells divide when they experience a relative increase of 50% in the levels of Dpp signaling. Since in this model, it is the relative differences rather than the absolute amount of Dpp signal that regulate cell divisions, the model can account for the uniform growth of the wing disc [86].

Despite being very simple and elegant, this model falls short in some aspects. Measurable changes in Dpp signaling levels most likely only occur in the medial disc since the Dpp gradient decays exponentially and extremely low levels are present in the lateral disc. As a result, the model can only account for uniform growth in the medial disc and not in the lateral portion of the disc. How the detection of temporal increases in the morphogen level is translated into cell divisions remains currently unknown.

Is growth really uniform in the wing imaginal disc? Labeling of S-phase cells with BrdU incorporation gives that impression (Fig. 2a). However, several studies have hinted that this was an oversimplification [91–93]. Recently, systematic quantification of clonal growth rates at different stages of wing disc development has revealed that cells at the center of the pouch proliferate slightly faster than the ones at the edges. This differential proliferation along the proximal-distal axis in the wing disc is maximal during the second instar larval stage and slowly equilibrates toward the end of larval development [94]. It remains to be determined whether this differential proliferation is due to differences in Dpp signaling levels.

3.2. Models in which Dpp is permissive for growth

The aforementioned models, that ascribe an instructive role to Dpp in driving disc growth, have been challenged by the work on *brk*, an important modulator of growth downstream of Dpp [22,24,29]. As mentioned previously, P-Mad/Med/Shn complexes directly repress *brk* transcription, thus limiting *brk* expression to the lateral disc [41,46] (Fig. 1). Animals carrying a hypomorphic allele of *brk*, in which detectable Brk protein expression is lost, survive to pupal stages and have wing discs with enlarged lateral regions suggesting that Brk inhibits growth laterally [22]. The group of Ginés Morata has shown that there is a negative correlation between Brk activity and the size of the disc and suggested that Brk may be the main target of Dpp for growth control [95]. The Basler group has further tested this hypothesis and has shown that when both *dpp* and *brk* are simultaneously removed, the tissue still overgrows and resembles *brk* mutants, which strongly support a growth model in which the graded distribution of neither Dpp nor Brk is a prerequisite for proliferation [90].

Lastly, patches of cells that are double mutant for either *tkv* and *brk*, or *mad* and *brk*, proliferate at a rate comparable to their neighbors which carry functional proteins of the Dpp signaling pathway [96,97]. Since cells lacking the Dpp receptor Tkv are blind to the changes in the extracellular Dpp levels, yet proliferate normally, the importance of changes in Dpp levels in actively driving cellular divisions was questioned [97]. These findings suggest that Dpp is more likely to play a permissive role in growth regulation by removing Brk from the medial disc and thus allowing these cells to proliferate. Interestingly, while ectopic Brk is very effective in inducing cell death in medial cells, naturally high Brk levels in lateral cells does not prohibit but only slightly reduces proliferation rates.

Establishing Brk as the main growth regulator downstream of Dpp raises the question as to how Brk regulates growth. Two potent growth promoters, *dmyc* and *bantam microRNA* (*ban*), have been shown to be direct Brk targets [98,99]. Dmyc drives cellular growth by promoting ribosome biogenesis [100], while Ban induces balanced cell growth and proliferation [101]. Dual repression of Dmyc and Ban activity may account for a large portion of growth regulation by Brk, but recent work has highlighted that two targets that are normally regarded as patterning targets of Brk, Spalt (Sal) and Optomotor-blind (Omb), may also contribute to cell survival and proliferation.

3.3. Sal and Omb are critical for epithelial integrity

The genes encoding the transcription factors Sal and Omb were the first targets identified to be regulated by Dpp signaling. Their nested expression patterns along the anterior–posterior axis served

as flagship examples for concentration-dependent responses to a morphogen. Both genes are expressed at and around the Dpp stripe within the pouch and *omb* can be induced at lower doses of Dpp activity than *sal* [3]. Their transcription is directly activated by P-Mad/Med complexes and directly repressed by Brk (Fig. 1b) [46,102]. Boundaries of Sal and Omb expression are informative for positioning of the wing veins L2 and L5 [103–106]. In addition to their role in patterning, Omb and Sal are also required for the growth regulatory function of Dpp. It is critical for medial cells to have the correct amount of Sal and Omb, as loss-of-function as well as gain-of-function clones of either gene round up and are eventually extruded from the epithelium, similar to what was described for Dpp receptor mutant cells previously [107–111]. It remains to be determined whether the cells are extruded due to differential expression of cell–cell adhesion molecules or to defects in cytoskeletal organization. The epithelial extrusion phenotype after clonal modifications to Dpp signaling is another curious feature of the medial cells that separates them from the lateral cells.

3.4. Sal and Omb in proliferation control

There are two redundant loci with similar expression patterns called *spalt major* (*salm*) and *spalt-related* (*salr*) that constitute the *sal* function [102]. Strikingly, Salm expression can partially rescue the small wing phenotype caused by ectopic Brk, suggesting that Salm might be an important Brk target in growth regulation [111]. Along the same lines, double knock-down of *salm*/*salr* efficiently rescues phenotypes associated with expression of TkvQD, a constitutively active version of Tkv. Somewhat confusingly however, patches of cells with extra Salm seem to have proliferation defects, round up and are extruded from the epithelia [111]. It is possible that Salm and Salr have opposing effects on proliferation and this could explain some of the discrepancy, but this remains a hypothesis to be tested. Nevertheless, while the mechanistic details are unknown, the work of Organista and De Celis highlights the importance of Sal function in epithelial integrity and proliferation control.

Zhang et al. have recently added an interesting twist to these findings and proposed that Dpp controls proliferation by an Omb-dependent regional control of *bantam* [112]. They observed that uniform expression of Omb induced ectopic cell divisions in the lateral cells and suppressed proliferation in the medial cells, generating a BrdU profile similar to that of uniform Dpp expression (Fig. 2b). Reducing Omb levels, however, promoted proliferation of the medial cells. These data prompted the authors to hypothesize that Omb blocks proliferation of the medial cells but drives it in the lateral cells, should it be expressed there. According to their model, these effects are mediated *via* differential regulation of *bantam*; Omb blocks *bantam* expression in the medial cells and can induce it laterally. How this opposite regulation of *bantam* is achieved remains unknown, but the lack of any conserved Omb binding sites in *bantam* enhancer suggested that it was indirect [112].

Surprisingly, suppressing Dpp pathway activity in the medial disc *via* expression of Dad, a potent inhibitor of the pathway, or *via* the repressor protein Brk, increased levels of BrdU incorporation in the medial cells (Fig. 2c). These results led to the argument that Dpp signaling actually blocks proliferation in the medial disc *via* Omb [112]. Hence, whether Dpp plays a positive or negative role in proliferation of medial cells became controversial. On the one hand, Dpp is clearly required to remove the repressor Brk from the medial cells, but on the other hand, reducing pathway activity seems to promote cell divisions. Notably, the proliferative response observed after Dad or Brk expression may be compensatory, as these treatments are known to induce cell death, a hypothesis that remains untested. Development of new approaches for precise quantification of growth parameters may help resolve this issue.

4. Dpp and scaling

Despite the wide ranges of body sizes individuals of a given species can have, the body plans are astoundingly reproducible. For example, from worms to humans, starvation leads to formation of smaller adults with proportionally smaller body parts. This invariance in proportions, despite the variance in absolute organ and body size, is called scaling. How scaling can be achieved has been actively investigated by computational scientists for a long time and several models have been developed [113–115]. However, quantitative experimental data that can support or disprove these models have started to emerge only recently.

One of the most beautiful demonstrations of scaling comes from a classical experiment referred to in many textbooks. In 1892, Hans Driesch halved 2–8 cell stage sea urchin embryos with the anticipation that each half would form half a sea urchin. However odd that might sound today, that was the expected result based on Weismann's theory of nuclear determination [116]. To Driesch's surprise, surviving halves went on to form smaller, but normal looking sea urchins or, in other words, the pattern scaled with size. More than 70 years later, working with hydra and sea urchins, Lewis Wolpert realized that the same pattern can be generated over a range of sizes, which reminded him of flags. And that is how the famous French Flag model of pattern formation came to life [117]. Wolpert started thinking of the problem of pattern formation and pattern scaling using the French Flag as a model. He hypothesized that a gradient of an instructive signal could provide an answer to this problem. If we assume that a certain concentration of a morphogen induces a given cell fate and, if the morphogen gradient can adjust to the growing tissue size, the pattern automatically scales to match the size (Fig. 3). Or, as Wolpert put it: "the flag comes in many sizes, but always the same pattern" [116].

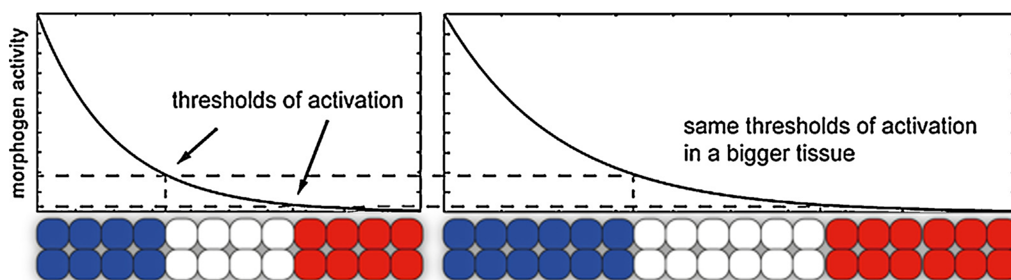

Fig. 3. Scaling the French Flag. A morphogen or its activity gradient provides positional information to the field. Different target genes are expressed at different concentration thresholds, establishing a pattern. In a bigger tissue where the gradient scales, the resulting pattern keeps the same proportions (1/3 of blue, white and red) and thus scales perfectly, provided that the boundaries are defined at the same threshold concentrations. Note that the concentrations at the morphogen source and at the end of the tissue are kept constant and the gradient is stretched to fit the larger size.

Reproduced from Hamaratoglu et al. [80].

Being the major factor that determines the pattern elements along the A/P axis in the wing, an important and obvious question to be asked is whether the Dpp gradient adjusts to the tissue size? And, if yes, is a certain cell fate always determined at a given absolute Dpp concentration?

Answering these questions requires a systems biology approach, in which high quality, quantitative data sets are generated and analyzed at different developmental stages during imaginal disc growth. Recently, three teams took up this challenge and showed that both the morphogen Dpp, as visualized by the Dpp-GFP fusion protein that is expressed in the Dpp stripe, and the cellular response to the Dpp gradient (visualized by P-Mad antibodies or GFP/RFP driven by a Dpp responsive *dad* enhancer) expand and adjust to the growing tissue size [80,81,86]. Hence, Dpp and its activity gradient scale with tissue size as the disc grows.

Importantly, while all three groups observed the ability of the Dpp or P-Mad gradients to scale with the tissue size, scaling was assessed using different criteria. Two groups defined scaling based on the French Flag model described in Fig. 3, and they showed that the P-Mad gradients scale with this definition [80,81]. As the disc grows, P-Mad gradients expand and become less steep while their amplitudes do not significantly change [80]. In contrast, Dpp-GFP levels were found to constantly increase in the medial disc [86]. If the Dpp levels indeed constantly increase in the tissue, the simplest version of the French Flag model-based decoding for determination of the boundaries of the Dpp target genes is ruled out; if the target gene expression domains scale with the tissues size, these boundaries would correspond to increasingly higher levels of the morphogen and hence would not be determined at constant concentration thresholds.

How can we reconcile steeply increasing Dpp levels during growth with roughly constant P-Mad levels? As a potential mechanism, it has been proposed that increases in Dad levels could counteract the increase in Dpp levels, since Dad is an inhibitory Smad [51,80]. Another possibility is that the system could get desensitized over time and more and more Dpp would be required to lead to similar P-Mad levels. Finally, it remains to be determined whether some of the increase in Dpp-GFP is due to the accumulation of the stable Gal4 protein in the tissue, since Gal4 was used to drive the expression of *UAS-Dpp-GFP*.

4.1. An expansion–repression mechanism for scaling the Dpp gradient

How do the Dpp and its activity gradients scale? A model developed in Naama Barkai's lab, named the expansion–repression integral feedback control, suggests that scaling arises as a spontaneous consequence of a feedback loop composed of a morphogen and a hypothetical molecule named expander. In this model, the expander helps the morphogen to spread, while having at the same time its own production suppressed by the morphogen [118]. Eventually, the morphogen reaches the sides of the tissue and the expander production is shut off. In the model, the expander molecule is stable and hence accumulates in the tissue, keeping the morphogen gradient extended. As the tissue grows, however, the lateral areas can again transcribe the expander and thereby further expand the morphogen gradient.

Consistent with the model predictions, two groups have identified the secreted molecule Pentagone (Pent) as an expander of Dpp in the wing disc. Indeed, Pent is required for the adjustment of the Dpp activity gradient to tissue size [80,81]. Moreover, Dpp suppresses *pent* transcription, restricting its production to the sides of the tissue [56]. Hence, it appears that secreted Pent molecules could move centrally and help Dpp reach further out, narrowing down the Pent-producing region. This model neatly accounts for the observed scaling behavior of the Dpp activity gradient. However, it is not yet quite perfect. Unlike in the model, high enough concentrations of Dpp never reaches the sides of the wing disc and *pent* expression persists [80]. While Pent does not seem to directly interact with Dpp, it may do so indirectly *via* binding the glypican Dally [56]. It was postulated that Pent may function as an expander by increasing the effective diffusion rate of Dpp or by decreasing its degradation rate. Either possibility could be realized by reduced affinity of Dpp to its receptor upon complex formation with Pent and Dally. Receptor binding prevents spreading of Dpp and may lead to its degradation *via* endocytosis [81].

Is the expansion–repression feedback loop, formed by Pent and Dpp, enough to account for most or all aspects of Dpp scaling? An interesting observation that came out of these analyses was that the scaling is not equally good at all positions in the field, suggesting that there might be extra measures taken to ensure scaling at critical positions. We asked whether the scaling of the Dpp activity gradient is transmitted to the expression domains of the downstream genes *sal* and *omb*. We found that while the Sal domain scaled very well in the anterior compartment, it hyperscaled – that is, it expanded more than required to match the tissue growth – in the posterior compartment. On contrary, the Omb domain scaled extremely well in the posterior compartment and poorly in the anterior compartment [80]. Notably, the anterior Sal domain boundary is important for the positioning of the longitudinal vein 2, while the posterior Omb domain boundary is necessary for the longitudinal vein 5 [104,119]. Thus, the Omb and Sal domain boundaries scale best with the tissue size where they have a known patterning function. It is tempting to speculate that this is not a mere coincidence. Considering their roles in vein positioning, to ensure the scaling of the anterior Sal domain and the posterior Omb domain would be essential.

In *pent* mutant discs, scaling of the posterior Omb domain is diminished and the adult wings lack the longitudinal vein 5. In this background, the anterior Sal domain exhibits good scaling in discs and the specification of vein 2 fate is not affected. Hence, the anterior Sal domain can scale in the absence of Pent function [80]. These results suggest that while Pent is clearly required for expansion of the Dpp gradient, it may not be the only expander or additional mechanisms may be at work to ensure proper scaling of downstream Dpp response.

4.2. Do constant thresholds determine gene expression domains?

Is the Dpp gradient interpreted *via* French-Flag decoding? Since the transcriptional regulation by Dpp signaling is executed by P-Mad and Brk, we asked whether the Omb and Sal domains respond to similar concentrations of P-Mad and Brk during disc growth [80]. In this case, provided that the activity gradients scale, the boundaries characterized by these constant thresholds would shift as the gradient expands, ensuring perfect scaling of the target gene domains with tissue size, as schematized in Fig. 3. We measured P-Mad and Brk concentrations at Sal and Omb boundaries during the third instar stage and found that with the exception of P-Mad at posterior Omb boundary, the values were variable. Hence, these boundaries do not appear to be set by simple French-Flag thresholds. However, since both P-Mad and Brk contribute to transcriptional regulation of the Dpp target genes (Fig. 1b), it is the combination of both signals that should remain constant, and not necessarily each signal individually. Significantly, it was possible to find functions with different combinations of P-Mad and Brk concentrations that were constant at these domain boundaries across development. For example, for Sal domain boundary in the anterior compartment, the multiplicative combination $\text{P-Mad}^5{*}\text{Brk}^4$ is constant, but it remains to be determined whether this has any biological significance or not [80].

Overall, these first attempts to quantitatively examine the Dpp signaling activity during development of the disc revealed many unexpected features and exciting hypotheses. Continuation of these efforts, where quantitative measurements are coupled to computational modeling, is likely to help us further understand how Dpp functions in growth control and scaling.

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References

[1] Spencer FA, Hoffmann FM, Gelbart WM. Decapentaplegic: a gene complex affecting morphogenesis in Drosophila melanogaster. Cell 1982;28:451–61.
[2] Padgett RW, St Johnston RD, Gelbart WM. A transcript from a Drosophila pattern gene predicts a protein homologous to the transforming growth factor-beta family. Nature 1987;325:81–4.
[3] Affolter M, Basler K. The Decapentaplegic morphogen gradient: from pattern formation to growth regulation. Nat Rev Genet 2007;8:663–74.
[4] Kicheva A, Gonzalez-Gaitan M. The Decapentaplegic morphogen gradient: a precise definition. Curr Opin Cell Biol 2008;20:137–43.
[5] O'Connor MB, Umulis D, Othmer HG, Blair SS. Shaping BMP morphogen gradients in the Drosophila embryo and pupal wing. Development 2006;133:183–93.
[6] Ramel MC, Hill CS. Spatial regulation of BMP activity. FEBS Lett 2012;586:1929–41.
[7] Raftery LA, Umulis DM. Regulation of BMP activity and range in Drosophila wing development. Curr Opin Cell Biol 2012;24:158–65.
[8] Ashe HL. BMP signalling: synergy and feedback create a step gradient. Curr Biol 2005;15:R375–7.
[9] Shravage BV, Altmann G, Technau M, Roth S. The role of Dpp and its inhibitors during eggshell patterning in Drosophila. Development 2007;134:2261–71.
[10] Yakoby N, Lembong J, Schüpbach T, Shvartsman SY. Drosophila eggshell is patterned by sequential action of feedforward and feedback loops. Development 2008;135:343–51.
[11] Xie T, Spradling AC. decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell 1998;94:251–60.
[12] Harris RE, Ashe HL. Cease and desist: modulating short-range Dpp signalling in the stem-cell niche. EMBO Rep 2011;12:519–26.
[13] Chen S, Wang S, Xie T. Restricting self-renewal signals within the stem cell niche: multiple levels of control. Curr Opin Genet Dev 2011;21:684–9.
[14] Pennetier D, Oyallon J, Morin-Poulard I, Dejean S, Vincent A, Crozatier M. Size control of the Drosophila hematopoietic niche by bone morphogenetic protein signaling reveals parallels with mammals. Proc Natl Acad Sci U S A 2012;109:3389–94.
[15] Li Z, Zhang Y, Han L, Shi L, Lin X. Trachea-derived dpp controls adult midgut homeostasis in Drosophila. Dev Cell 2013;24:133–43.
[16] Li H, Qi Y, Jasper H. Dpp signaling determines regional stem cell identity in the regenerating adult Drosophila gastrointestinal tract. Cell Rep 2013;4:10–8.
[17] Nellen D, Burke R, Struhl G, Basler K. Direct and long-range action of a DPP morphogen gradient. Cell 1996;85:357–68.
[18] Lecuit T, Brook WJ, Ng M, Calleja M, Sun H, Cohen SM. Two distinct mechanisms for long-range patterning by Decapentaplegic in the Drosophila wing. Nature 1996;381:387–93.
[19] Müller P, Rogers KW, Yu SR, Brand M, Schier AF. Morphogen transport. Development 2013;140:1621–38.
[20] Parker L, Stathakis DG, Arora K. Regulation of BMP and activin signaling in Drosophila. Prog Mol Subcell Biol 2004;34:73–101.
[21] Pyrowolakis G, Hartmann B, Affolter M. BMP and Activin Signaling in Drosophila. In: Miyazono RDa K, editor. The TGF-B Family. 2008.
[22] Campbell G, Tomlinson A. Transducing the Dpp morphogen gradient in the wing of Drosophila: regulation of Dpp targets by brinker. Cell 1999;96:553–62.
[23] Jaźwińska A, Kirov N, Wieschaus E, Roth S, Rushlow C. The Drosophila gene brinker reveals a novel mechanism of Dpp target gene regulation. Cell 1999;96:563–73.
[24] Minami M, Kinoshita N, Kamoshida Y, Tanimoto H, Tabata T. brinker is a target of Dpp in Drosophila that negatively regulates Dpp-dependent genes. Nature 1999;398:242–6.
[25] Upadhyai P, Campbell G. Brinker possesses multiple mechanisms for repression because its primary co-repressor, Groucho, may be unavailable in some cell types. Development 2013;140:4256–65.
[26] Winter SE, Campbell G. Repression of Dpp targets in the Drosophila wing by Brinker. Development 2004;131:81.
[27] Hasson P, Müller B, Basler K, Paroush Z. Brinker requires two corepressors for maximal and versatile repression in Dpp signalling. EMBO J 2001;20:5725–36.
[28] Affolter M, Marty T, Vigano MA, Jazwinska A. Nuclear interpretation of Dpp signaling in Drosophila. EMBO J 2001;20:3298–305.
[29] Jazwinska A, Rushlow C, Roth S. The role of brinker in mediating the graded response to Dpp in early Drosophila embryos. Development 1999;126:3323–34.
[30] Ashe HL, Mannervik M, Levine M. Dpp signaling thresholds in the dorsal ectoderm of the Drosophila embryo 2000;. Development 2000;127:3305–12.
[31] Lin MC, Park J, Kirov N, Rushlow C. Threshold response of C15 to the Dpp gradient in Drosophila is established by the cumulative effect of Smad and Zen activators and negative cues. Development 2006;133:4805–13.
[32] Rushlow C, Colosimo PF, Lin MC, Xu M, Kirov N. Transcriptional regulation of the Drosophila gene zen by competing Smad and Brinker inputs. Genes Dev 2001;15:340–51.
[33] Xu M, Kirov N, Rushlow C. Peak levels of BMP in the Drosophila embryo control target genes by a feed-forward mechanism. Development 2005;132:1637–47.
[34] Markstein M, Zinzen R, Markstein P, Yee KP, Erives A, Stathopoulos A, et al. A regulatory code for neurogenic gene expression in the Drosophila embryo. Development 2004;131:2387–94.
[35] Wharton SJ, Basu SP, Ashe HL. Smad affinity can direct distinct readouts of the embryonic extracellular Dpp gradient in Drosophila. Curr Biol 2004;14:1550–8.
[36] Zhang H, Levine M, Ashe HL. Brinker is a sequence-specific transcriptional repressor in the Drosophila embryo. Genes Dev 2001;15:261–6.
[37] Ashe HL, Briscoe J. The interpretation of morphogen gradients. Development 2006;133:385–94.
[38] Chen H, Xu Z, Mei C, Yu D, Small S. A system of repressor gradients spatially organizes the boundaries of Bicoid-dependent target genes. Cell 2012;149:618–29.
[39] Gafner L, Dalessi S, Escher E, Pyrowolakis G, Bergmann S, Basler K. Manipulating the sensitivity of signal-induced repression: quantification and consequences of altered brinker gradients. PLoS ONE 2013;8:e71224.
[40] Marty T, Muller B, Basler K, Affolter M. Schnurri mediates Dpp-dependent repression of brinker transcription. Nat Cell Biol 2000;2:745–9.
[41] Muller B, Hartmann B, Pyrowolakis G, Affolter M, Basler K. Conversion of an extracellular Dpp/BMP morphogen gradient into an inverse transcriptional gradient. Cell 2003;113:221–33.
[42] Yao LC, Phin S, Cho J, Rushlow C, Arora K, Warrior R. Multiple modular promoter elements drive graded brinker expression in response to the Dpp morphogen gradient. Development 2008;135:2183–92.
[43] Torres-Vazquez J, Park S, Warrior R, Arora K. The transcription factor Schnurri plays a dual role in mediating Dpp signaling during embryogenesis. Development 2001;128:1657–70.
[44] Arora K, Dai H, Kazuko SG, Jamal J, O'Connor MB, Letsou A, et al. The Drosophila schnurri gene acts in the Dpp/TGF beta signaling pathway and encodes a transcription factor homologous to the human MBP family. Cell 1995;81:781–90.
[45] Grieder NC, Nellen D, Burke R, Basler K, Affolter M. Schnurri is required for Drosophila Dpp signaling and encodes a zinc finger protein similar to the mammalian transcription factor PRDII-BF1. Cell 1995;81:791–800.
[46] Pyrowolakis G, Hartmann B, Muller B, Basler K, Affolter M. A simple molecular complex mediates widespread BMP-induced repression during Drosophila development. Dev Cell 2004;7:229–40.
[47] Staehling-Hampton K, Laughon AS, Hoffmann FM. A Drosophila protein related to the human zinc finger transcription factor PRDII/MBPI/HIV-EP1 is required for dpp signaling. Development 1995;121:3393–403.
[48] Moser M, Campbell G. Generating and interpreting the Brinker gradient in the Drosophila wing. Dev Biol 2005;286:647–58.
[49] Gao S, Steffen J, Laughon A. Dpp-responsive silencers are bound by a trimeric Mad-Medea complex. J Biol Chem 2005;280:36158–64.
[50] Weiss A, Charbonnier E, Ellertsdottir E, Tsirigos A, Wolf C, Schuh R, et al. A conserved activation element in BMP signaling during Drosophila development. Nat Struct Mol Biol 2010;17:69–76.
[51] Tsuneizumi K, Nakayama T, Kamoshida Y, Kornberg TB, Christian JL, Tabata T. Daughters against dpp modulates dpp organizing activity in Drosophila wing development. Nature 1997;389:627–31.
[52] Gao S, Laughon A. Flexible interaction of Drosophila Smad complexes with bipartite binding sites. Biochim Biophys Acta 2007;1769:484–96.
[53] Crocker J, Erives A. A Schnurri/Mad/Medea complex attenuates the dorsal-twist gradient readout at vnd. Dev Biol 2013;378:64–72.
[54] Garcia M, Stathopoulos A. Lateral gene expression in Drosophila early embryos is supported by Grainyhead-mediated activation and tiers of dorsally-localized repression. PLoS ONE 2011;6:e29172.
[55] Clark RI, Woodcock KJ, Geissmann F, Trouillet C, Dionne MS. Multiple TGF-β superfamily signals modulate the adult Drosophila immune response. Curr Biol 2011;21:1672–7.
[56] Vuilleumier R, Springhorn A, Patterson L, Koidl S, Hammerschmidt M, Affolter M, et al. Control of Dpp morphogen signalling by a secreted feedback regulator. Nat Cell Biol 2010;12:611–7.
[57] Szuperák M, Salah S, Meyer EJ, Nagarajan U, Ikmi A, Gibson MC. Feedback regulation of Drosophila BMP signaling by the novel extracellular protein larval translucida. Development 2011;138:715–24.
[58] Ogiso Y, Tsuneizumi K, Masuda N, Sato M, Tabata T. Robustness of the Dpp morphogen activity gradient depends on negative feedback regulation by the inhibitory Smad. Dad Dev Growth Differ 2011;53:668–78.

[59] Sawala A, Sutcliffe C, Ashe HL. Multistep molecular mechanism for bone morphogenetic protein extracellular transport in the Drosophila embryo. Proc Natl Acad Sci U S A 2012;109:11222–7.
[60] Shimmi O, Umulis D, Othmer H, O'Connor MB. Facilitated transport of a Dpp/Scw heterodimer by Sog/Tsg leads to robust patterning of the Drosophila blastoderm embryo. Cell 2005;120:873–86.
[61] Wang YC, Ferguson EL. Spatial bistability of Dpp-receptor interactions during Drosophila dorsal-ventral patterning. Nature 2005;434:229–34.
[62] Wang X, Harris RE, Bayston LJ, Ashe HL. Type IV. collagens regulate BMP signalling in Drosophila. Nature 2008;455:72–7.
[63] Gavin-Smyth J, Wang YC, Butler I, Ferguson EL. A genetic network conferring canalization to a bistable patterning system in Drosophila. Curr Biol 2013;23:2296–302.
[64] Liang HL, Xu M, Chuang YC, Rushlow C. Response to the BMP gradient requires highly combinatorial inputs from multiple patterning systems in the Drosophila embryo. Development 2012;139:1956–64.
[65] Tanimoto H, Itoh S, ten Dijke P, Tabata T. Hedgehog creates a gradient of DPP activity in Drosophila wing imaginal discs. Mol Cell 2000;5:59–71.
[66] Lecuit T, Cohen SM. Dpp receptor levels contribute to shaping the Dpp morphogen gradient in the Drosophila wing imaginal disc. Development 1998;125:4901–7.
[67] Crickmore MA, Mann RS. Hox control of organ size by regulation of morphogen production and mobility. Science 2006;313:63–8.
[68] Makhijani K, Kalyani C, Srividya T, Shashidhara LS. Modulation of Decapentaplegic gradient during haltere specification in Drosophila. Dev Biol 2007;302:243–55.
[69] de Navas LF, Garaulet DL, Sánchez-Herrero E. The ultrabithorax Hox gene of Drosophila controls haltere size by regulating the Dpp pathway. Development 2006;133:4495–506.
[70] Xia L, Jia S, Huang S, Wang H, Zhu Y, Mu Y, et al. The Fused/Smurf complex controls the fate of Drosophila germline stem cells by generating a gradient BMP response. Cell 2010;143:978–90.
[71] Akiyama T, Kamimura K, Firkus C, Takeo S, Shimmi O, Nakato H. Dally regulates Dpp morphogen gradient formation by stabilizing Dpp on the cell surface. Dev Biol 2008;313:408–19.
[72] Belenkaya TY, Han C, Yan D, Opoka RJ, Khodoun M, Liu H, et al. Drosophila Dpp morphogen movement is independent of dynamin-mediated endocytosis but regulated by the glypican members of heparan sulfate proteoglycans. Cell 2004;119:231–44.
[73] Fujise M, Takeo S, Kamimura K, Matsuo T, Aigaki T, Izumi S, et al. Dally regulates Dpp morphogen gradient formation in the Drosophila wing. Development 2003;130:1515–22.
[74] Han C, Belenkaya TY, Wang B, Lin X. Drosophila glypicans control the cell-to-cell movement of Hedgehog by a dynamin-independent process. Development 2004;131:601–11.
[75] Jackson SM, Nakato H, Sugiura M, Jannuzi A, Oakes R, Kaluza V, et al. dally, a Drosophila glypican, controls cellular responses to the TGF-beta-related morphogen, Dpp. Development 1997;124:4113–20.
[76] Yan D, Wu Y, Feng Y, Lin SC, Lin X. The core protein of glypican Dally-like determines its biphasic activity in wingless morphogen signaling. Dev Cell 2009;17:470–81.
[77] Takei Y, Ozawa Y, Sato M, Watanabe A, Tabata T. Three Drosophila EXT genes shape morphogen gradients through synthesis of heparan sulfate proteoglycans. Development 2004;131:73–82.
[78] Vuilleumier R, Affolter M, Pyrowolakis G. Pentagone: patrolling BMP morphogen signaling Fly (Austin) 2011;5:210–4.
[79] Zheng Q, Wang Y, Vargas E, DiNardo S. magu is required for germline stem cell self-renewal through BMP signaling in the Drosophila testis. Dev Biol 2011;357:202–10.
[80] Hamaratoglu F, de Lachapelle AM, Pyrowolakis G, Bergmann S, Affolter M. Dpp signaling activity requires pentagone to scale with tissue size in the growing Drosophila wing imaginal disc. PLoS Biol 2011;9:e1001182.
[81] Ben-Zvi D, Pyrowolakis G, Barkai N, Shilo BZ. Expansion-repression mechanism for scaling the Dpp activation gradient in Drosophila wing imaginal discs. Curr Biol 2011;21:1391–6.
[82] Burke R, Basler K. Dpp receptors are autonomously required for cell proliferation in the entire developing Drosophila wing. Development 1996;122:2261–9.
[83] Zecca M, Basler K, Struhl G. Sequential organizing activities of engrailed, hedgehog and decapentaplegic in the Drosophila wing. Development 1995;121:2265–78.
[84] Schwank G, Basler K. Regulation of organ growth by morphogen gradients. Cold Spring Harb Perspect Biol 2010;2:a001669.
[85] Restrepo S, Zartman JJ, Basler K. Coordination of Patterning and Growth by the Morphogen DPP. Curr Biol 2014;24:R245–55.
[86] Wartlick O, Mumcu P, Kicheva A, Bittig T, Seum C, Julicher F, et al. Dynamics of Dpp signaling and proliferation control. Science 2011;331:1154–9.
[87] Rogulja D, Irvine KD. Regulation of cell proliferation by a morphogen gradient. Cell 2005;123:449–61.
[88] Day SJ, Lawrence PA. Measuring dimensions: the regulation of size and shape. Development 2000;127:2977–87.
[89] Martin-Castellanos C, Edgar BA. A characterization of the effects of Dpp signaling on cell growth and proliferation in the Drosophila wing. Development 2002;129:1003–13.
[90] Schwank G, Restrepo S, Basler K. Growth regulation by Dpp: an essential role for Brinker and a non-essential role for graded signaling levels. Development 2008;135:4003–13.
[91] Gonzalez-Gaitan M, Capdevila MP, Garcia-Bellido A. Cell proliferation patterns in the wing imaginal disc of Drosophila. Mech Dev 1994;46:183–200.
[92] Milan M, Campuzano S, Garcia-Bellido A. Cell cycling and patterned cell proliferation in the wing primordium of Drosophila. Proc Natl Acad Sci U S A 1996;93:640–5.
[93] O'Brochta DA, Bryant PJ. A zone of non-proliferating cells at a lineage restriction boundary in Drosophila. Nature 1985;313:138–41.
[94] Mao Y, Tournier AL, Hoppe A, Kester L, Thompson BJ, Tapon N. Differential proliferation rates generate patterns of mechanical tension that orient tissue growth. EMBO J 2013;32:2790–803.
[95] Martin FA, Perez-Garijo A, Moreno E, Morata G. The brinker gradient controls wing growth in Drosophila. Development 2004;131:4921–30.
[96] Schwank G, Dalessi S, Yang SF, Yagi R, de Lachapelle AM, Affolter M, et al. Formation of the long range Dpp morphogen gradient. PLoS Biol 2011;9:e1001111.
[97] Schwank G, Yang SF, Restrepo S, Basler K. Comment on "Dynamics of dpp signaling and proliferation control". Science 2012;335:401, author reply 401.
[98] Oh H, Irvine KD. Cooperative regulation of growth by Yorkie and Mad through bantam. Dev Cell 2011;20:109–22.
[99] Doumpas N, Ruiz-Romero M, Blanco E, Edgar B, Corominas M, Teleman AA. Brk regulates wing disc growth in part via repression of Myc expression. EMBO Rep 2013;14:261–8.
[100] Johnston LA, Gallant P. Control of growth and organ size in Drosophila. Bioessays 2002;24:54–64.
[101] Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 2003;113:25–36.
[102] de Celis JF, Barrio R, Kafatos FC. A gene complex acting downstream of dpp in Drosophila wing morphogenesis. Nature 1996;381:421–4.
[103] de Celis JF, Barrio R. Function of the spalt/spalt-related gene complex in positioning the veins in the Drosophila wing. Mech Dev 2000;91:31–41.
[104] Cook O, Biehs B, Bier E. brinker and optomotor-blind act coordinately to initiate development of the L5 wing vein primordium in Drosophila. Development 2004;131:2113–24.
[105] Sturtevant MA, Biehs B, Marin E, Bier E. The spalt gene links the A/P compartment boundary to a linear adult structure in the Drosophila wing. Development 1997;124:21–32.
[106] Biehs B, Sturtevant MA, Bier E. Boundaries in the Drosophila wing imaginal disc organize vein-specific genetic programs. Development 1998;125:4245–57.
[107] Milán M, Pérez L, Cohen SM. Short-range cell interactions and cell survival in the Drosophila wing. Dev Cell 2002;2:797–805.
[108] Gibson MC, Perrimon N. Extrusion and death of DPP/BMP-compromised epithelial cells in the developing Drosophila wing. Science 2005;307:1785–9.
[109] Shen J, Dahmann C. Extrusion of cells with inappropriate Dpp signaling from Drosophila wing disc epithelia. Science 2005;307:1789–90.
[110] Shen J, Dahmann C, Pflugfelder GO. Spatial discontinuity of optomotor-blind expression in the Drosophila wing imaginal disc disrupts epithelial architecture and promotes cell sorting. BMC Dev Biol 2010;10:23.
[111] Organista MF, De Celis JF. The Spalt transcription factors regulate cell proliferation, survival and epithelial integrity downstream of the Decapentaplegic signalling pathway. Biol Open 2013;2:37–48.
[112] Zhang X, Luo D, Pflugfelder GO, Shen J. Dpp signaling inhibits proliferation in the Drosophila wing by Omb-dependent regional control of bantam. Development 2013;140:2917–22.
[113] Lander AD, Pattern. growth, and control. Cell 2011;144:955–69.
[114] Umulis DM, Othmer HG. Mechanisms of scaling in pattern formation. Development 2013;140:4830–43.
[115] Ben-Zvi D, Shilo BZ, Barkai N. Scaling of morphogen gradients. Curr Opin Genet Dev 2011;21:704–10.
[116] Wolpert L, Beddington R, Brockes J, Jessell T, Lawrence P, Meyerowitz E. Principles of development. Oxford University Press; 1998.
[117] Wolpert L. Diffusible gradients are out - an interview with Lewis Wolpert. Interviewed by Richardson. Michael K Int J Dev Biol 2009;53:659–62.
[118] Ben-Zvi D, Barkai N. Scaling of morphogen gradients by an expansion-repression integral feedback control. Proc Natl Acad Sci U S A 2010;107:6924–9.
[119] De Celis JF. Pattern formation in the Drosophila wing: The development of the veins. Bioessays 2003;25:443–51.