Understanding morphogenetic growth control — lessons from flies

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Abstract | Morphogens are secreted signalling molecules that control the patterning and growth of developing organs. How morphogens regulate patterning is fairly well understood; however, how they control growth is less clear. Four principal models have been proposed to explain how the morphogenetic protein Decapentaplegic (DPP) controls the growth of the wing imaginal disc in the fly. Recent studies in this model system have provided a wealth of experimental data on growth and DPP gradient properties, as well as on the interactions of DPP with other signalling pathways. These findings have allowed a more precise formulation and evaluation of morphogenetic growth models. The insights into growth control by the DPP gradient will also be useful for understanding other morphogenetic growth systems.

Imaginal disc
A flat epithelial pouch in the larva that, during metamorphosis in the pupa, will give rise to an adult structure of the fly.

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How the growth and patterning of developing tissues are controlled and coordinated has been a long-standing question in developmental biology. Morphogens are secreted signalling molecules that play a part in these processes by providing positional information to the cells in the tissue and by acting as a trigger for tissue growth (reviewed in REFS 1–3). Different morphogen concentrations induce distinct target gene responses; therefore, in a concentration gradient, the cellular response is position dependent, resulting in tissue patterning. How morphogens regulate tissue growth is less clear.

An excellent model system for the study of morphogenetic growth regulation is the wing imaginal disc of *Drosophila melanogaster*, in which tissue growth is controlled by the morphogen Decapentaplegic (DPP)[4] (FIG. 1a,b). DPP is a bone morphogenetic protein (BMP) homologue and triggers the activation of known wing patterning genes in a concentration-dependent manner[5–9] (FIG. 1b). Binding of DPP to its receptor Thickveins (TKV) leads to the phosphorylation and activation of the transcription factor MAD[10,11]. Phosphorylated MAD in turn represses the expression of Brinker (BRK), which is itself a transcriptional repressor[12–15]. Together, phosphorylated MAD and BRK regulate the expression of DPP target genes[16] (FIG. 1c).

Although it is clear that DPP regulates growth[4], for a long time no direct growth-regulatory targets of DPP were known. However, recently it was found that phosphorylated MAD interacts with the co-transcriptional activator Yorkie to regulate the transcription of the growth-promoting microRNA (miRNA) *bantam* (FIG. 1c). Even so, absolute DPP signalling levels cannot directly control proliferation; this is because in the disc cells are exposed to different absolute DPP levels but proliferate at the same rate[17–19] (BOX 1; FIG. 1b). Several models for growth control by DPP have been proposed to address this issue[4].

These morphogenetic growth models fall into two groups. In the first, DPP is permissive for growth: DPP levels above a certain threshold promote growth in the central region of the disc, whereas growth in the periphery, where DPP levels are below the threshold, is regulated by mechanical stress[20–22] or other growth factors[17,18]. In the second, DPP is instructive for growth: to set their growth rate or cell cycle length, cells are proposed to measure a position-independent DPP gradient property, such as spatial differences in DPP signalling levels between neighbouring cells[23,24] or the increase in cellular DPP signalling levels over time[24].

Recent studies have provided new data on DPP gradient and wing disc growth properties and the relationship between them[24]. Here, we review these data and discuss how far they support the proposed morphogenetic growth models.

Wing disc growth and the DPP gradient

The growth properties of the wing imaginal disc have been well characterized (BOX 1). During the larval growth period, cells undergo approximately ten divisions. Cells initially proliferate quickly, but the proliferation rate decreases as development proceeds. At any one time during development, all of the cells, no matter where they

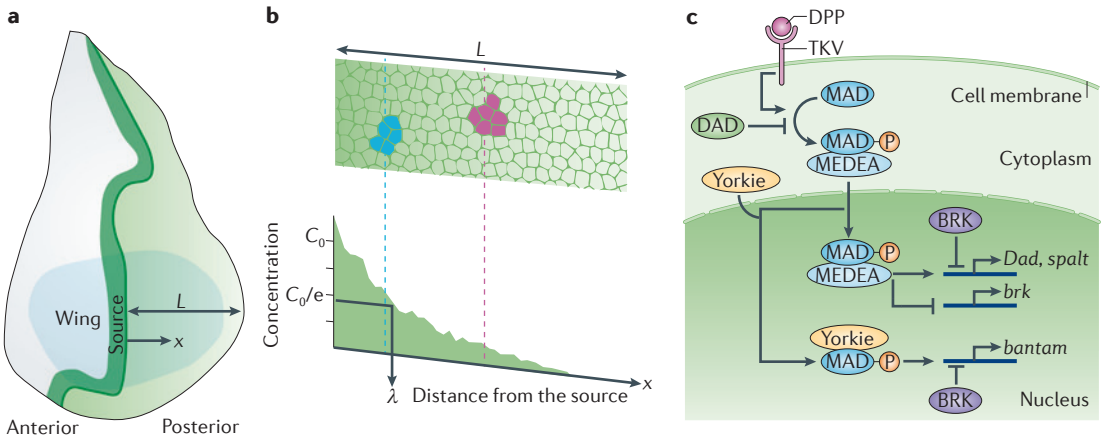


Figure 1 | **The Decapentaplegic gradient. a** | The imaginal wing disc of *Drosophila melanogaster*. The compartment boundary (green line), Decapentaplegic (DPP) source and gradient in the tissue (green; here the gradient is only shown in the posterior target compartment) are shown. *L* indicates target tissue width, and *x* the distance from the source. **b** | Magnified view of cells in the posterior compartment and the DPP gradient (top), with an intensity profile (bottom) as a function of the distance from the source; the gradient is characterized by its amplitude (C_0) and decay length (λ). Note that growth is approximately homogeneous: two clones (blue and pink) at different positions in the gradient have grown to the same size; however, because the clones are exposed to different DPP concentrations they express different target genes (blue and pink). **c** | The DPP signalling pathway. The transcription factor MAD is phosphorylated and activated following binding of DPP to its receptor Thickveins (TKV). MAD can then bind MEDEA and accumulates in the nucleus, where it represses the transcription of the repressor *brinker* (*brk*). BRK and phosphorylated MAD regulate DPP target genes, such as *Dad* and *spalt*. Phosphorylated MAD also interacts with the co-transcriptional activator Yorkie to regulate the transcription of the growth-promoting microRNA *bantam*. MAD phosphorylation is negatively regulated by DAD.

are in the tissue, divide at roughly the same rate[17,18,24,25]. In other words, all cells contribute approximately equally to the tissue: tissue proportions scale. This means that the position of cell clones relative to the width of the tissue remains approximately constant during development (FIG. 2a). Similar observations have been made in other morphogenetic growth systems, such as the vertebrate limbs or the embryonic spinal chord (BOX 1). Below, we discuss how the DPP concentration gradient changes during wing disc growth.

DPP gradient scaling during wing disc growth. DPP is expressed in a central stripe of cells (the DPP source) and spreads in the tissue, forming a concentration gradient[6,7,26] (FIG. 1a). The DPP gradient can be characterized by its amplitude (C_0) and its decay length (λ)[27] (FIG. 1b). Quantification of the amplitude and decay length of the DPP gradient in the posterior compartment of the wing disc showed that the DPP gradient expands during disc growth[24] (FIG. 2a,b). In particular, the decay length is proportional to the width of the compartment — the gradient scales (FIG. 2c,d). Gradient scaling means that, as the tissue grows, the range of the gradient grows proportionately. Therefore, when the relative gradient profiles are normalized to tissue size, gradients from all stages of development have the same shape[24] (FIG. 2c).

An important implication of this invariant relative concentration profile is that, during development, a particular relative position in the tissue always has the same relative concentration of the morphogen[24] (C/C_0 = constant; FIG. 2c). Because homogeneous growth means that cells (and their lineages) do not change their relative position during growth (FIG. 2a), gradient scaling implies that a particular cell always experiences the same relative DPP concentration as the tissue grows[24] (C_{cell}/C_0 = constant). This means that, for the observed increase in the gradient amplitude in the growing tissue (FIG. 2b,e), there is a corresponding increase of cellular DPP concentration: C_{cell} and C_0 are proportional in all cells. In other words, the DPP signalling levels of all cells in the tissue increase over time by the same percentage as the gradient amplitude (FIG. 2e).

Gradient scaling is not only observed for the DPP ligand concentration gradient, but also for the DPP signalling gradient[24]. The transduction of the signal involves successive nonlinear amplifications[24]. This means that the spatial activity pattern of the pathway components — for example, phosphorylated MAD, BRK and DAD — is different to that of DPP ligand concentration[24]. However, gradient scaling and the increase of the amplitude are also observed for a downstream DPP signalling 'readout' (*Dad* tanscription)[24]. This is not trivial, and it will be interesting to understand how the wiring of the signal transduction network mediates the conservation of these two properties at the end of the pathway.

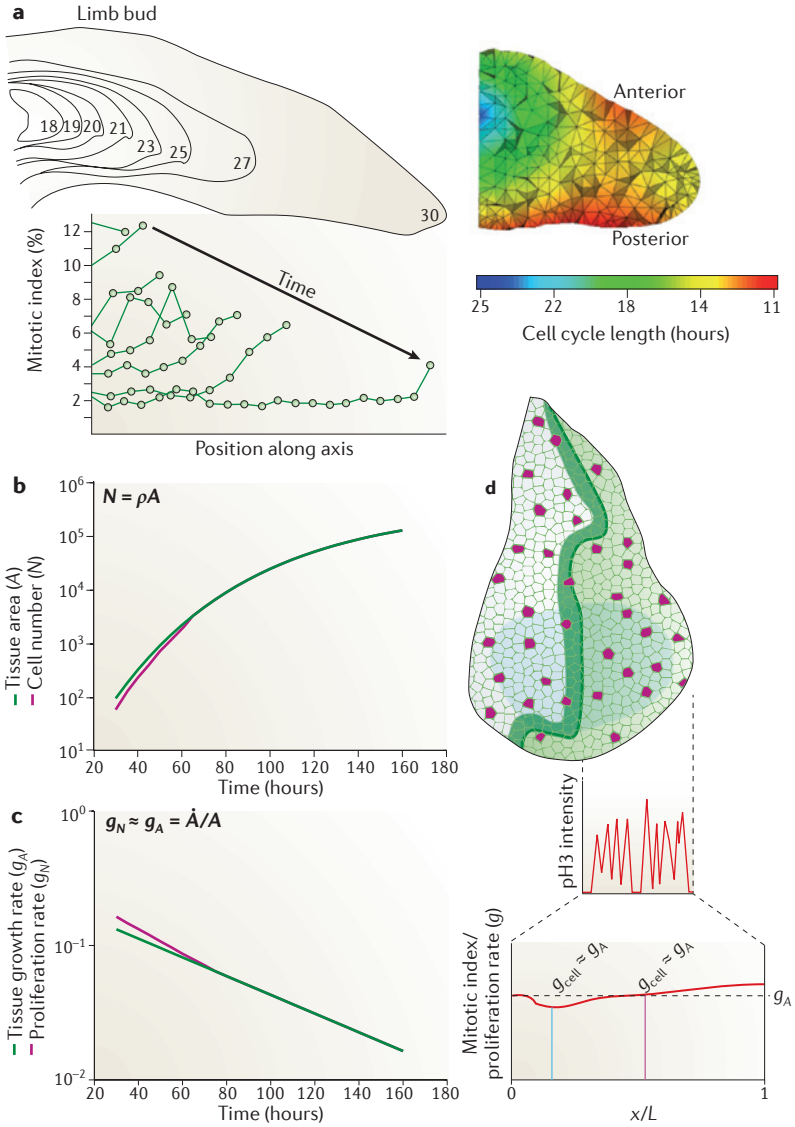
Controlling the DPP gradient during wing disc growth. Disc growth does not distort the DPP gradient because the gradient renews itself faster than the tissue grows[24]. Gradient scaling is therefore not a direct consequence of growth. Instead, scaling is mainly due to a decrease in the DPP degradation rate — empirically, when the number of cells in the tissue doubles, the DPP degradation rate halves[24]. Both the increase of the gradient amplitude C_0 and the scaling of the decay length λ can be explained as a consequence of this decrease of the DPP degradation

Amplitude
The maximum concentration of a protein in the target region. In the case of Decapentaplegic (DPP), amplitude refers to the concentration at the DPP source boundary. Its value depends on DPP production and degradation rates, as well as on the number of DPP-producing cells (the source width) and on the rate of diffusion.

Decay length
A measure for the spatial range of a protein gradient (how far it reaches into the tissue): the position at which the concentration of the protein is a fixed fraction of its amplitude (C_0/e; in which e is Euler's number). Its value depends on how fast the molecules diffuse and are degraded (less degradation equals a higher decay length).

Gradient scaling
If the gradient expands at the same rate as the tissue, it scales with tissue size; the decay length of the gradient is proportional to the width of the tissue.

Box 1 | **Tissue growth properties**



The wing imaginal disc of *Drosophila melanogaster* displays growth properties similar to those of many other developmental systems. First, like in the vertebrate limbs or the embryonic spinal chord, morphogen gradients seem to control growth[4,79]. Second, like in vertebrate systems, the growth rate decreases during development and is fairly homogeneous in space[24,80–82] (some heterogeneities are apparent, but they are relatively small and confined to the boundary regions). This was described in the classical paper by Hornbruch and Wolpert in 1970 (REF. 82), as well as more recently with quantitative detail by the Sharpe group[80] (see the figure, part **a**, which shows growth in a chick limb bud (left) and a mouse limb bud (right)).

However, vertebrate limb systems grow as mesenchymal masses of cells (which are three-dimensional (3D)), whereas imaginal discs are epithelia (2D sheets); this reduces the analysis of their growth to a 2D problem. The measurement of disc growth properties is therefore simple. The cell density (ρ) in disc epithelia increases slightly during development. Because the increase in cell density is much smaller than the increase in cell number (N) (threefold versus 1,000-fold[24,43]), the tissue area (A) is roughly proportional to the number of cells (see the figure, part **b**); cell number = cell density × tissue area ($N = \rho A$) As a result, the average cell doubling time is equal to the area doubling time (see the figure, part **c**). The height of cells along the z axis increases slightly as the cell density increases[83,84], so that overall the average cell volume is approximately constant (O.W., unpublished observations). Indeed, cell growth and proliferation are coordinated in wing disc cells — cells divide when they have doubled their volume[85–87]. Furthermore, apoptosis levels are low and fairly uniform[88]. Therefore, the average cell growth rate and proliferation rate (g_N) are roughly the same and correspond to the effective tissue growth rate (g_A). Finally, staining with 5-bromodeoxyuridine (BrdU) and phosphorylated histone H3 (pH3) (used to visualize proliferation and cell division) as well as clonal analyses suggest that growth is fairly homogeneous[17–19,24,25,50]. This implies that all cells have the same growth rate (g_{cell}), which is approximately equal to the average tissue growth rate g_A (see the figure, part **d**) and can therefore be inferred directly from measurements of the disc area. L, tissue width; x, distance from the morphogen source. Part **a** of the figure is modified, with permission, from REFS 80,82.

rate during development (see Supplementary information S1 (table)). A key question, therefore, is how tissue growth causes the decrease of the DPP degradation rate. Molecules regulating morphogen degradation and thereby expanding a gradient in response to growth have been termed expanders[28]. Below, the mechanisms by which an expander might control DPP degradation are referred to as scaling mechanisms.

Two types of scaling mechanisms have been proposed (FIG. 3). An expander mechanism based on expansion–repression feedback was originally proposed by Ben-Zvi and Barkai[28–30]. In this model, the expander antagonizes DPP degradation. The expander is a long-lived, rapidly diffusible molecule, the expression of which is repressed above a certain DPP concentration level. When the imaginal disc grows, cellular DPP levels at the edge of the disc

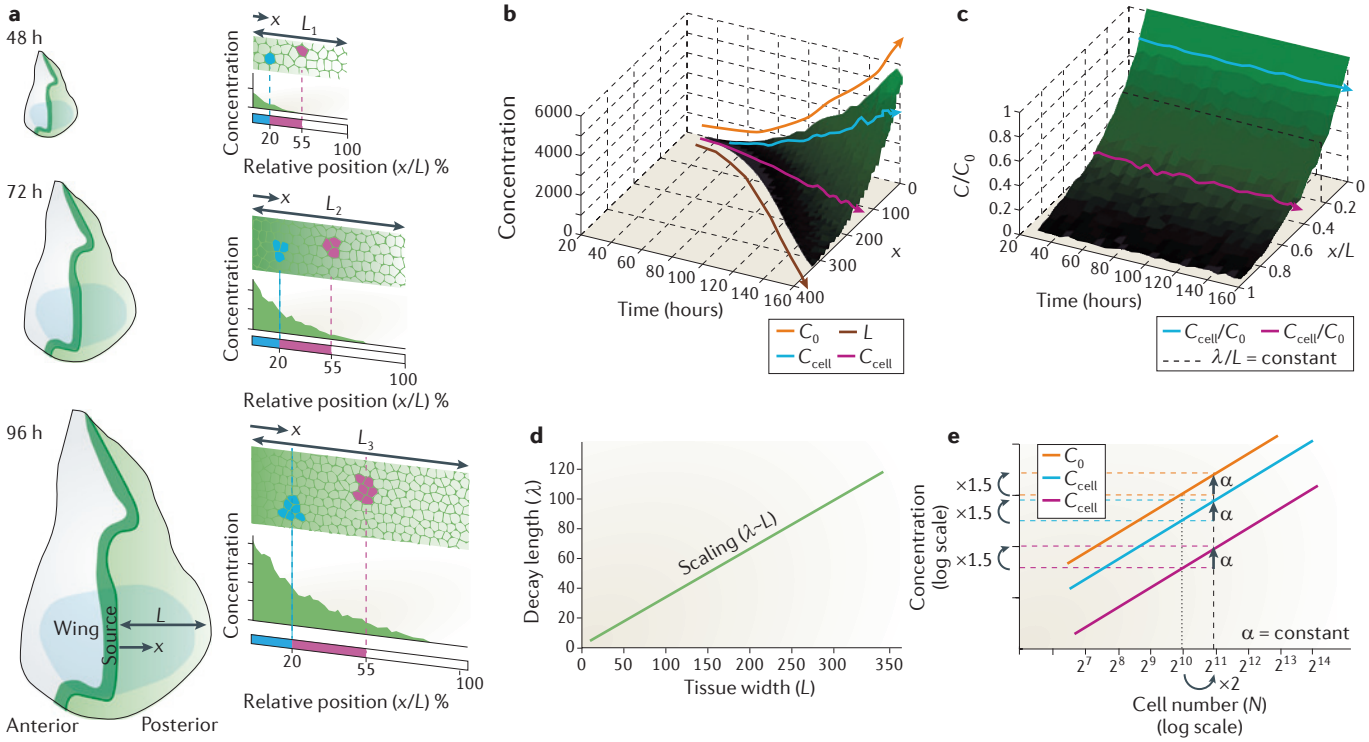


Figure 2 | **Decapentaplegic gradient properties during growth. a** | The Decapentaplegic (DPP) gradient expands in growing wing imaginal discs; homogeneous growth implies that tissue proportions, and therefore the relative position (distance from the source/tissue width (*x*/*L*)) of cells and their lineage, stay constant during development (two clones, blue and pink, are shown). **b**–**e** | Gradient scaling implies that the DPP concentration profiles at different times of development (**b**) collapse onto the same shape when relative concentration (C/C_0; in which C_0 is the gradient amplitude) and relative position (*x*/*L*) are considered (**c**). This implies that the ratio of gradient decay length to tissue width (λ/L) is constant during development (in other words, λ is proportional to *L* (**d**)), and cells at certain relative positions (blue and pink) always see the same relative DPP concentration (C_{cell}/C_0) (**c**). This, in turn, implies that DPP concentration experienced by a particular cell (C_{cell}) is proportional to C_0. The relative increase in concentration over time is the same for all cells (**e**) and empirically correlates with growth: whenever the cellular concentration increases by a percentage α, the number of cells doubles (**e**).

fall below the threshold and cells start expressing the expander (FIG. 3a). The expander then diffuses in the tissue and decreases the DPP degradation rate, thus expanding the DPP concentration gradient. This results in an increase in DPP levels at the edge of the disc and repression of the expander, accompanied by disc growth[28,30].

The second type of scaling mechanism is based on reducing the DPP degradation rate in response to cell division events[24]. This can be achieved, for example, by cell-autonomous dilution of a long-lived expander that promotes DPP degradation and might also diffuse in the tissue (FIG. 3b). In this expander-dilution mechanism, the degradation rate is inversely proportional to the number of cells and, by extension, the area of the tissue, so the gradient scales with the square root of the tissue area but not necessarily with the tissue width (FIG. 3b; see Supplementary information S1 (table)). By contrast, in the expansion–repression mechanism, the gradient does not necessarily scale with tissue area but scales with the width of the tissue in the direction of the gradient, as repression of the expander directly depends on the DPP gradient along this axis (FIG. 3a).

Therefore, anisotropic tissue growth could help distinguish between the two types of scaling mechanism (see Supplementary information S1 (table)). Indeed, analysis of DPP gradient scaling in different anisotropic growth conditions showed that area scaling, but not width scaling, was the same in the different experimental conditions[24], suggesting that gradient scaling might be due to a dilution-type mechanism, rather than expansion–repression.

So far, no molecular equivalent of an expander has been identified. However, Pentagone (PENT), a molecule that is conserved from flies to vertebrates[31], shows some of the features required of an expander: it is secreted, diffusible and repressed by DPP signalling (and therefore transcribed in regions of low DPP concentration)[31]. PENT antagonizes DPP degradation by interacting with the heparan sulphate proteoglycan Division abnormally delayed (DALLY)[31], which itself is involved in DPP turnover and diffusion[32–35]. It has yet to be determined whether DPP gradient scaling is modified in *pent* mutants.

An obvious target for an expander in the dilution mechanism would be DPP receptor levels: the expander could affect the effective intracellular DPP degradation rate by modulating DPP receptor levels through receptor ubiquitylation and internalization or through changes in endosomal dynamics. The TKV degradation rate, like the DPP degradation rate, seems to decrease

Anisotropic tissue growth
Directionally dependent growth of a tissue: more growth along one axis (for example, horizontal) than another (for example, vertical) in the plane of the epithelium.

during development[24], so it would be interesting to pursue this line of investigation further.

Finally, it is unlikely that the scaling of the signalling gradient happens only at the level of the ligand concentration gradient. Indeed, in experiments in which both endogenous *dpp* and its downstream target *brk* (FIG. 1c) are mutated, an overall increase in the transcription of the DPP target gene *Dad* was still observed during development[24]. Increased transcription of *Dad* over time even in the absence of DPP and the BRK repressor might indicate that not only DPP degradation but also target gene production is sensitive to tissue size. Alternative explanations are that a second BMP-type morphogen, GBB, can activate MAD and also contribute to *Dad* transcription[36–39], or that the mutations used here might not represent complete loss of functions.

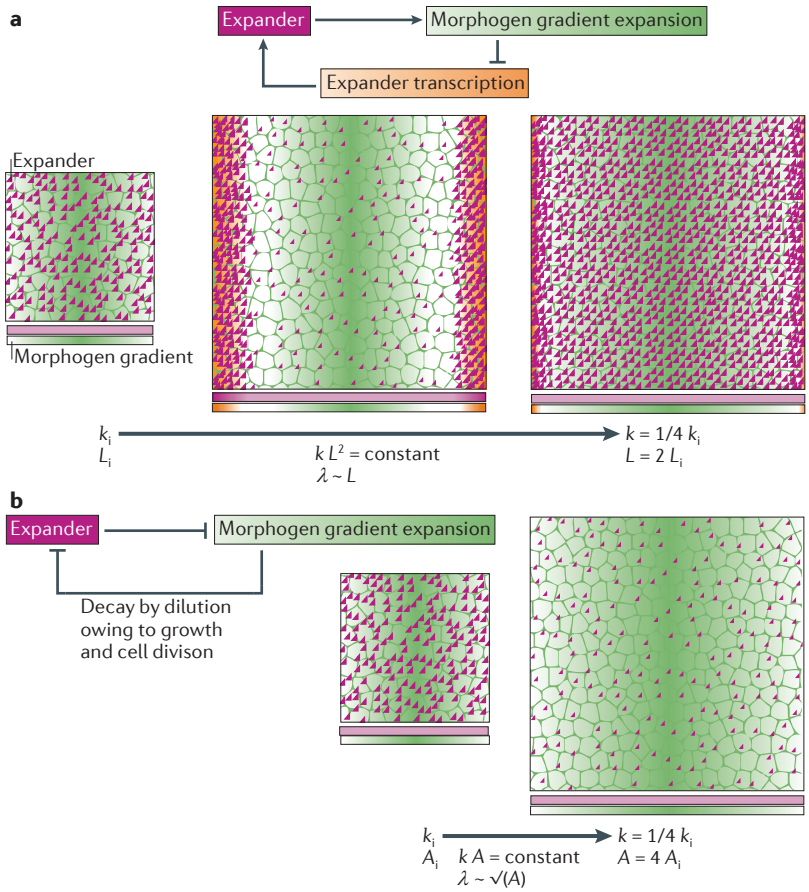


Figure 3 | **Possible mechanisms for morphogen gradient expansion and scaling.** **a** | Expansion–repression mechanism. The expander (pink) antagonizes morphogen degradation. It is transcribed (orange) below a threshold morphogen concentration (green); when tissue growth causes cells in the periphery to experience morphogen levels below this threshold, they produce a long-lived expander that diffuses in the tissue, reduces morphogen degradation rates (k) and thereby expands the morphogen gradient to fit into the new tissue width (L). In this model, the initial degradation rate k_i decreases in an inversely proportional manner to L^2; this implies that the gradient decay length (λ) is proportional to the tissue width L. This is known as width scaling. **b** | Expander-dilution mechanism. The expander promotes degradation of the morphogen. When the tissue grows, the expander is diluted in response to cell division events, and, therefore, morphogen degradation rates (k) decrease and the morphogen gradient expands. In this model, the degradation rate k is inversely proportional to the tissue area A; this implies that the gradient decay length λ is proportional to the square root of the tissue area A. This is known as area scaling (see also Supplementary information S1 (table)).

Growth control by the DPP gradient

The previous section considered how growth affects the DPP gradient. But, how does the DPP gradient influence growth control in the imaginal disc? Proliferation is approximately spatially uniform and decreases over time (BOX 1). By contrast, DPP concentration and signalling levels are graded in space and increase over time (FIG. 2). Therefore, absolute DPP levels alone cannot control homogeneous proliferation. However, cells could interpret spatial differences[23,24] or temporal changes in DPP concentration or signalling levels[24] to set their growth rate or cell cycle length. Another possibility is that additional factors, such as mechanical stress or the presence of other growth factors, act in concert with DPP to make growth homogeneous (FIG. 4).

Below, we discuss the four major proposed morphogenetic growth control models: growth control by mechanical feedback, growth control by complementary inhibition and growth control based on spatial differences in DPP levels between neighbouring cells or on temporal changes in DPP signalling.

Growth control based on mechanical feedback. In epithelia, cells adjust their height and apical surface area in response to mechanical stress (for example, compression by surrounding cells). In other words, high levels of mechanical stress can physically limit tissue growth by locally limiting cell growth[22,40]. The idea that mechanical stress limits cell growth led to the formulation of models in which DPP is merely permissive for growth (that is, it promotes growth when its levels are above a certain threshold) and mechanical stress acts as a long-range signal to instruct cell growth rates[20–22].

In a simple model based on mechanical feedback, proliferation is stimulated by a central point source of growth factors, generated by the intersection of line sources of the two diffusible morphogens, DPP and Wingless, in the centre of the disc[20–22]. Cells divide above a certain threshold of growth factor concentration. Proliferation forces cells to move out of the central growth factor zone into the periphery, where they can divide easily even with low growth factor levels because they experience low mechanical stress levels[20–22]. Proliferation in the periphery then compresses cells in the centre and limits their growth[20–22]. Thus, the build-up of mechanical stress distributions in the tissue can lead to a situation in which growth is homogeneous even when there is a steep DPP gradient. As cell compression, and thus mechanical stress, increases during development, the effective tissue growth rate decreases overall[20–22] (FIG. 4a).

Experimental support for the role of mechanical stress in growth control comes from measurements of cell compression in the centre of the disc[41,42], which suggest that central cell compression increases during development[41]. Whether this increase in mechanical stress is large enough to be limiting for cell growth is not clear.

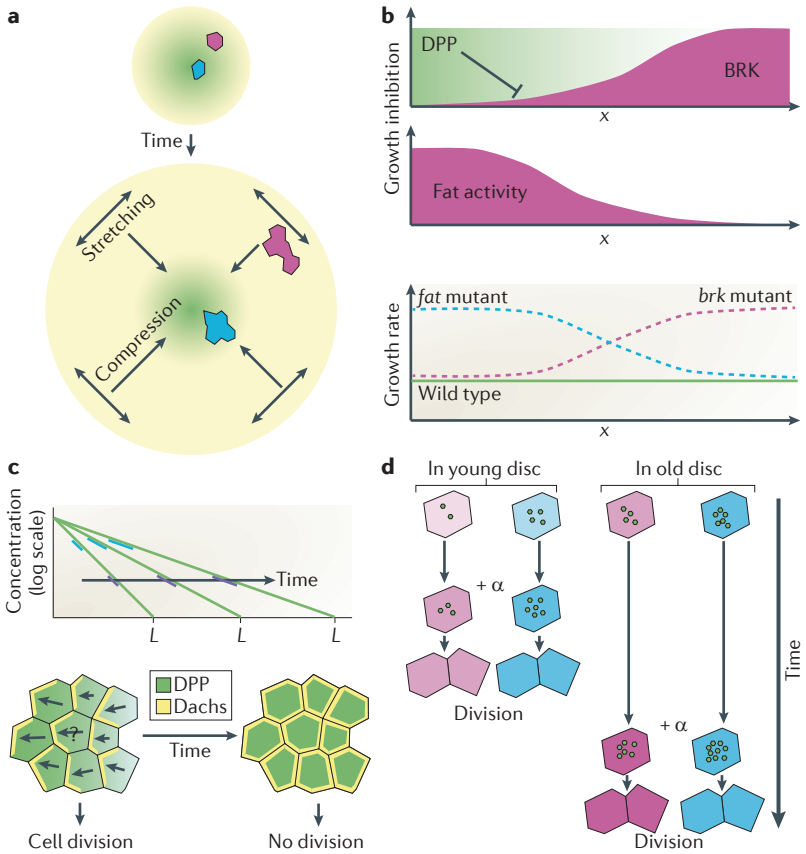


Figure 4 | **Models for growth control by morphogen gradients. a** | Mechanical stress model: the proliferation of cells in the central region in response to a morphogen (green) stretches the cells in the periphery, causing them to divide (low stress; yellow). The proliferation of cells in the periphery compresses the cells in the centre, inhibiting their growth. Growth factors (morphogens) and low stress (stretching) stimulate division, whereas compression inhibits growth. This model assumes that gradient scaling does not occur, and the shape of the gradient is irrelevant. **b** | Complementary inhibitor model. In this model, the gradient is irrelevant. Decapentaplegic (DPP) allows growth by repressing Brinker (BRK) in the central region of the disc; in addition, Fat activity controls growth in a similar manner at the edge of the disc. The spatial profile of the Fat activity gradient (top) has not been measured directly but is inferred from the growth phenotype of *fat* mutants (bottom). This model requires that the DPP and Fat activity gradients are established in an independent manner. *x* indicates distance from the morphogen source. **c** | Spatial model. The relative slope of an exponential morphogen concentration gradient could control cell division (top). For a scaling gradient, the relative slope decreases as the tissue grows, consistent with decreasing growth rates; disc cells (bottom) can sense relative spatial differences in DPP (green) (and probably other factors) through the non-conventional myosin Dachs (yellow). Large spatial differences in DPP levels lead to cell division. By contrast, cell division would not occur when the DPP concentration gradient is uniform. **d** | Temporal model. Growth is controlled by a percentage increase in morphogen concentration (of $\alpha = 50\%$) over time (morphogen concentration is illustrated by the number of dots in each cell). The time it takes to reach a 50% increase in concentration is equal to the cell cycle length and is longer at late stages of development because the increase in concentration becomes smaller as the tissue grows.

In fact, area growth does not seem to be constrained by the increase in cell compression: the tissue area increases substantially even late in development, when the cell density no longer changes[24,43], indicating that mechanical stress levels in the disc may not directly control or significantly limit cell growth rates.

Furthermore, some assumptions made by mechanical growth models do not seem to apply to the wing imaginal disc. First, data suggest that Wingless does not control growth in the wing disc[44,45], so the growth factor (DPP) is produced by a line source rather than a point source. A line source causes different stress and proliferation patterns than a point source because there is no longer a peripheral ring of stretched, proliferating cells that compresses the cells in the central growth factor region. As a result, proliferation would (probably) not be uniform.

Another assumption in the original model was that the growth factor gradient does not scale[21,22]. For a scaling gradient such as DPP, the central growth factor region would simply expand as the tissue grows. As a consequence, cells would not move out of this zone into the periphery, there would be no compression of cells in the central region, and growth would not stop.

A mechanical model based on modified assumptions might still provide an explanation for the growth properties of the wing imaginal disc. However, the specific form of such a modified model is currently unclear. In any case, a systematic experimental analysis of stress distributions in the disc at different times of development is needed to get a clearer picture of the possible role of mechanical stresses as regulators of proliferation.

Growth control by complementary inhibition. Another permissive growth model proposed that DPP allows growth by suppressing BRK, an integral component of the DPP pathway (FIG. 4b). BRK is a repressor of DPP target genes and is repressed by DPP signalling; it is therefore expressed as an 'anti-gradient' (REFS 12,13,15) (FIG. 1c). The proposal for a permissive role of DPP in growth through the repression of BRK was based on the observation that, although *tkv*-mutant or *Mad*-mutant cells do not grow, cells mutated for both TKV and BRK or for both MAD and BRK do grow[12–14]. Thus, high levels of BRK inhibit growth, and mutation of BRK rescues the growth of cells lacking DPP signalling upstream of BRK (FIG. 1c). Therefore, BRK plays a major part in DPP signal transduction and the downstream regulation of growth.

Consistent with these observations, wing discs in which BRK is ubiquitously expressed do not grow, *brk*-mutant discs overgrow in lateral regions (where BRK is normally expressed) and, strikingly, wing discs that are deficient in both DPP and BRK have a growth phenotype similar to *brk* mutants[25]. Because BRK acts as a repressor in the DPP pathway, the output of the pathway manifested in target gene expression was assumed to be maximal in *brk*-mutant cells[4,25,46]. As a result, DPP signalling levels were assumed to be spatially uniform in a *brk*-mutant disc[4,25,46]. Thus, the observation that in *brk*-mutant discs cells in lateral regions proliferate more than cells in the centre (although they are supposedly experiencing the same DPP signalling levels) implied that growth in central and lateral regions of the disc had to be regulated independently and that the growth role of DPP in the centre is just permissive: the gradient is irrelevant for growth in the centre[25].

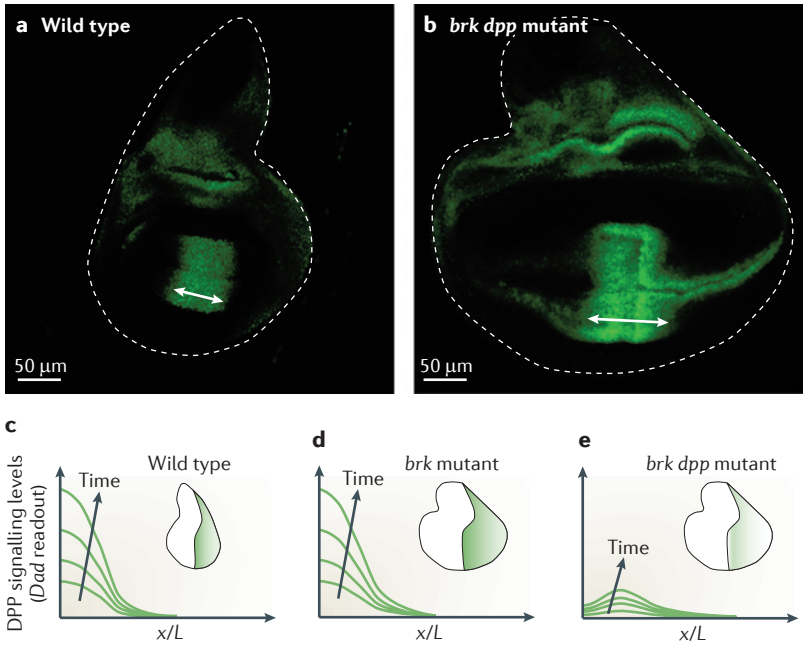


Figure 5 | **The role of BRK in signal transduction and growth. a**,**b** | Decapentaplegic (DPP) target gene *spalt* (green) in a wild-type imaginal disc (**a**) and in a *brinker* (*brk*) and *dpp* double mutant (**b**). Note that the expression pattern and range of *spalt* (white arrow) is similar in both the wild type and the *brk dpp* mutant; note also that, although there is ectopic expression, the expression of *spalt* is not spatially uniform in *brk dpp* double mutants. This indicates that the output of the DPP signalling pathway downstream of BRK is not uniform in space because otherwise *spalt* should be uniformly expressed in the entire disc. **c**–**e** | DPP signalling levels (graphs) and growth phenotypes (insets) in wild-type imaginal discs (**c**), *brk* mutants (**d**) and *brk dpp* double mutants (**e**) based on quantifications of red fluorescent protein expression, driven by the *Dad* enhancer[24]. DPP signalling levels are not spatially uniform and they increase over time; they are also more than tenfold lower in *brk dpp* mutants than in *brk* mutants and wild-type cells (not drawn to scale here). *L* indicates tissue width and *x* distance from the morphogen source. Images in parts **a** and **b** are modified, with permission from REF. 25 © (2008) The Company of Biologists.

At first, it was hypothesized that growth in the periphery is controlled by mechanical stress[20,21] (see above). More recently, it was proposed that homogeneous growth could be regulated by an additional growth inhibitory gradient that is complementary to the inhibitory BRK gradient[46]; specifically, the activity gradient of Fat, which is involved in growth mediated by the Hippo pathway (see below) (FIG. 4b). This proposal was based on the growth phenotype of *fat*-mutant wing imaginal discs, which is complementary to that of *brk* mutants[46] (FIG. 4b). Furthermore, overgrowth of imaginal discs with increased DPP signalling levels could be suppressed by increasing Fat activity[46].

In the complementary inhibitor model, the fact that BRK and Fat activity are graded in space is not relevant for growth regulation, as long as the shape of the gradients is perfectly complementary. However, for this model it is crucial that the two gradients are independent[46,47]. If they are not — for example, if Fat activity is downstream of DPP — then there is no complementary inhibition, but instead growth depends only on DPP signalling. Indeed, DPP signalling affects the expression of Dachsous and Four-jointed, two proteins regulating Fat activity, and the localization of Dachs, a protein responding to Fat activity[48]. It is difficult to reconcile these experimental findings with the complementary inhibition model. Independent establishment of two exactly complementary gradients is also problematic because it is difficult to ensure robustness. In any case, however, factors other than Fat could still regulate growth in a manner complementary to BRK.

The key assumption of this model is that DPP signalling levels and the expression of target genes is maximal in *brk*-mutant cells and, therefore, spatially uniform in *brk*-mutant discs, which overgrow[4,25,46]. In other words, in the absence of a DPP signalling pattern in space (a spatially uniform signal) and of changes in time (a maximal signal), growth still occurs: thus, DPP is just permissive for growth. However, although DPP target genes (such as *spalt* and *Dad*) are ectopically expressed in *brk*-mutant clones of cells and *brk*-mutant discs, their expression is not spatially uniform[12,13,15,24,25] (FIG. 5). This indicates that loss of repression by BRK does not lead to maximum DPP signalling. Indeed, analysis of the *Dad* enhancer revealed parallel inputs into the *Dad* regulatory regions from BRK and MAD, rather than BRK alone[16]. Therefore, graded signalling in *brk* mutants could, in principle, be due to BRK-independent signalling mediated by MAD. This does not seem to be the case, however, because in *brk dpp* double mutants, which lack phosphorylated MAD, the expression of target genes is also not spatially uniform[24,25] (FIG. 5). This reveals that our understanding of the DPP signalling pathway is incomplete, because our current model of the pathway (FIG. 1c) would predict spatially and temporally uniform DPP target gene expression in the absence of both BRK and phosphorylated MAD.

Strikingly, although absolute levels of DPP output, as monitored with a *Dad* transcriptional reporter, are much lower in the *brk dpp* mutant (FIG. 5e), cells still proliferate, indicating that the absolute output of the DPP signalling pathway downstream of BRK cannot directly determine the growth rate (although one cannot completely exclude the possibility that the low DPP signalling levels are still above a permissive threshold). However, because the DPP signalling output in *brk dpp* mutant conditions is neither uniform in space nor constant in time[24,25] (FIG. 5), growth of *Mad brk* mutant or *tkv brk* mutant cells[12–14] is not necessarily uncovering a scenario in which DPP is merely permissive for growth. The growth phenotypes of these mutants might also be consistent with instructive growth models, in which cells measure either spatial differences or temporal changes in DPP signalling levels to set their growth rate or cell cycle length.

Growth control based on spatial differences in DPP signalling. One of the first instructive models proposed that proliferation could be controlled by the local slope of the DPP gradient: neighbouring cells measure this slope through their differences in DPP levels, the slope flattens during the growth phase, and proliferation stops when the slope reaches a minimal steepness[23] (FIG. 4c). This model was first proposed by Day and Lawrence[23], who assumed that there is a linear gradient that stretches as the tissue grows. This would explain homogeneous

growth (a single slope in a linear gradient) and the slow-down of the growth rate (as the gradient becomes shallower during growth, its slope becomes smaller).

Regeneration experiments in cockroaches supported this hypothesis[49]. However, the model was challenged by a mosaic analysis in *D. melanogaster* wing discs carrying clones of cells that express a constitutively active form of TKV (TKV^{QD}) and therefore have higher DPP signalling levels than surrounding cells[19]. According to the model, cells at the edge of such a clone would experience a big spatial difference in DPP signalling levels and should therefore divide more. No extra proliferation was observed at the border of clones[19], and this observation was thought to refute the model. However, recent experiments showed that there is a transient additional proliferation effect on the cells at the border of clones, observed only in a time-course experiment[50]. The transient extra proliferation of cells exposed to big spatial differences in DPP signalling levels supports the idea that spatial cues have a role in growth control.

The DPP gradient is not a linear gradient with a constant slope in space, as had been assumed by Day and Lawrence in the first spatial model; instead, it is steeper close to the source and shallow away from it[27,28,51] (FIG. 1a). In other words, the slope of the DPP gradient is actually position dependent and so cannot control position-independent proliferation rates. However, the exponential approximation of the DPP gradient[26,27,50] shows that relative spatial differences in DPP concentration — that is, the percentage by which the DPP concentration decreases across a cell's surface, or C'/C (the spatial derivative of C, normalized to C) — are, like the growth rate, position independent. In an exponential gradient, C'/C is the same for all the cells in the tissue at a given time and depends only on one global parameter, the decay length λ, which increases as the tissue grows[24] ($C'/C = 1/\lambda$; for details, see supplementary information S1 (table)). Therefore, the value of C'/C is the same for all cells, and, like their growth rate, C'/C decreases during development.

One way to directly test whether growth is controlled by spatial cues would be to create uniform DPP signalling in space. Wing discs in which *brk* is mutated or DPP is ubiquitously expressed overgrow[25]. It was thought that, if spatial differences in DPP levels between cells were the driving force of proliferation, there should have been less growth. However, the overgrowth phenotype is not inconsistent with a spatial model because, on closer inspection, the expression of DPP targets was unexpectedly not uniform in these conditions, indicating a non-uniform DPP signalling profile[24,25] (FIG. 5). Indeed, later quantifications showed that, even when DPP is ubiquitously expressed, relative spatial differences in signalling levels between neighbouring cells still correlate with their growth rates[24]. However, this correlation is less clear in the *brk* mutant[24]. It is also important to note that the parameters of the spatial model (for example, the C'/C threshold below which cells stop dividing at the end of the growth phase) differed between different tissues and mutant conditions[24]. Nevertheless, the spatial model remains a plausible mechanism for growth control.

Growth control based on temporal changes in DPP signalling. An alternative to the spatial model proposes that cell cycle length is determined by relative temporal changes in cellular DPP levels; that is, by the percentage by which the DPP concentration increases over time, or $\dot{C}/C$ (the time derivative of C, normalized to C) (FIG. 4d). Empirically, it was found that when DPP signalling levels have increased by a percentage α (in this case α = 50%) from the beginning of the cell cycle, cells divide[24] (FIG. 2e). If the gradient scales, cellular DPP signalling levels increase by the same percentage in all cells (FIG. 2e), and therefore, in a temporal model, all cells have the same growth rate[24] (see Supplementary information S1 (table)). If DPP signalling levels increase quickly, α is reached quickly, and the cell proliferation rate is high (corresponding to a short cell cycle length). At the end of development, when DPP levels increase more slowly (FIG. 2b), it takes cells a longer time to reach α, and therefore the cell cycle is longer (FIG. 4d).

Support for the temporal model came from experiments in which the velocity of the temporal increase of DPP signalling was manipulated exogenously by drug-mediated induction of the expression of TKV^{QD} in clones of cells. In this experiment, the DPP signalling levels in clone cells increased faster than in wild-type cells; however, consistent with a temporal model, the cells divided when DPP signalling levels had increased by α = 50%[24]. How quickly cells attained this 50% increase depended on the concentration of the drug that determined the velocity of the increase in DPP signalling levels. It also depended on how distant the clones were from the source: clones far away from the source initially had lower DPP signalling levels. Therefore, upon TKV^{QD} expression, the relative increase in their DPP signalling levels was bigger. Because of this, clones that were away from the source were bigger than those closer to the source, which is consistent with the temporal growth model[19,24].

Interestingly, growth control based on the temporal model can account for non-homogeneous proliferation patterns observed in and around TKV^{QD} clones and in imaginal discs in which DPP signalling is ubiquitously increased[24]. Under these conditions, the gradient no longer scales, and, as a consequence, DPP signalling levels increase faster or slower depending on position: unlike in wild-type discs, $\dot{C}/C$ is position dependent. Importantly, cell proliferation rates are also position dependent and correlate with the position-dependent $\dot{C}/C$[24]. This further supports the temporal model as a plausible mechanism for growth control.

Measurement of C′/C or Ċ/C by cells. In models in which DPP is merely permissive for growth (FIG. 4a,b), the relevance and output of DPP signalling is fairly easy to understand: cells above a certain threshold of DPP concentration would express DPP growth targets (for example, the miRNA *bantam*) and divide. By contrast, how cells could measure relative spatial differences or temporal changes in DPP signalling levels is much less intuitive. It is easy to show that these gradient properties correlate with the growth rate, but how could cells actually measure relative changes in concentration in space or over time?

Spatial derivative
The spatial difference in a quantity (for example, concentration) across one spatial unit (for example, a cell); the spatial derivative is denoted with a prime symbol, for example $dC / dx = C'$.

Time derivative
The rate of change of a quantity (for example, concentration) over time; the time derivative is denoted with a dot symbol, for example $dC / dt = \dot{C}$.

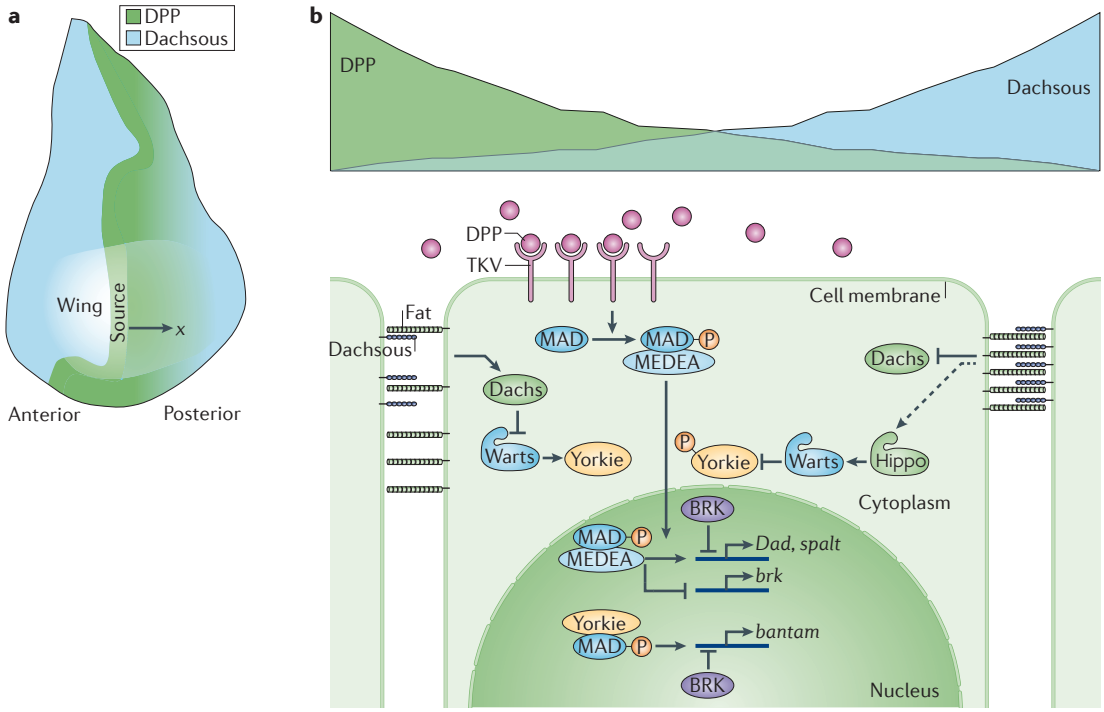


Figure 6 | **Interaction between Decapentaplegic and the Fat–Hippo pathway. a** | Decapentaplegic (DPP; green) forms a gradient along the anterior–posterior axis (here the gradient is only shown in the posterior compartment to the right). Dachsous (blue) forms a gradient along the proximo–distal axis (it is high along the edges and decreases towards the centre). *x* indicates distance from the morphogen source. **b** | The DPP pathway and the Fat signalling pathway responding to Dachsous interact. High levels of Dachsous (right) activate the Fat signalling pathway, inhibiting Dachs and Yorkie (through the kinase Warts). By contrast, on the side of the cell where Dachsous levels are low (left) — which can be caused by an ectopic increase in DPP signalling levels in clones of cells — Dachs is active, leading to the activation of Yorkie. Together with phosphorylated MAD, Yorkie regulates the transcription of the growth target *bantam*. The arrows shown do not indicate regulatory pathways but outcomes. The dashed arrow indicates an indirect interation. BRK, Brinker; TKV, Thickveins.

To measure relative spatial differences in DPP, cells must compare signalling levels at different locations to each other and with respect to their own signalling levels. A related phenomenon might occur during planar cell polarity (PCP), when local levels of PCP factors in neighbouring cells can influence intracellular gradients of such factors[52]. In the wing disc, cells do indeed seem to be able to measure DPP signalling differences between adjacent cells using the Fat–Hippo pathway[48] (FIG. 6). This signalling pathway regulates organ size by controlling cell growth, proliferation and apoptosis[53]. The ligand for Fat is the protocadherin Dachsous, which forms a proximo–distal gradient in the disc[48,54,55] (FIG. 6a). Cells respond to the Fat signalling gradient by differential intracellular activation of the non-conventional myosin Dachs[48,55–57]: Dachs accumulates at the cell–cell interface that is facing the lower levels of Dachsous (FIG. 6b). This also corresponds to the cell–cell interface at which DPP (and Wingless) signalling levels are higher. When the spatial pattern of DPP signalling was changed, for example by generating clones of cells expressing TKV^{QD} or BRK, Dachs relocalized to the new interface facing higher DPP signalling levels[48]. The amount of Dachs at the interface seems to be independent of absolute DPP levels, suggesting that cells may sense relative spatial differences in DPP signalling levels[48].

Dachs regulates the activity of Yorkie by inactivating Warts, a kinase that reduces Yorkie activity (FIG. 6b). Yorkie regulates the expression of growth regulatory targets, such as Cyclin E, *D. melanogaster* inhibitor of apoptosis 1 and MYC, as well as the growth-promoting miRNA *bantam*[53,58–65] (the last in cooperation with the DPP transcription factor MAD[66]). Although the quantitative relationships between the Fat–Hippo pathway and the DPP gradient are still obscure, an intriguing possibility is that this pathway could detect spatial discontinuities in the DPP gradient and in response regulate the elimination of these discontinuities by local upregulation of cell proliferation or apoptosis, through titration of active Dachs and Yorkie levels. Such a mechanism might be responsible for regeneration in response to tissue damage[67,68], the perturbation of growth observed in and around TKV^{QD}- or BRK-expressing clones[50], and even the phenomenon of cell competition, when slow-growing clones with low DPP signalling levels are eliminated from the tissue[69,70].

Measurement of the relative temporal changes of signalling levels implies that adaptive responses are generated in the signalling pathway (reviewed in REF. 71). An adaptive response allows cells to measure relative increases of a signal, because the signalling system adapts to ambient concentrations of the signal and increasing

Planar cell polarity
A mechanism of cellular organization, distinct from apical–basal polarity, by which cells acquire information about their orientation within the tissue in the plane of the epithelium.

concentrations are necessary to maintain the response[72]. In other words, such signalling systems can sense an increase with respect to previous levels of the signal. Adaptive signalling pathways have been extensively studied in other model systems in which relative changes of a signal are measured, such as chemotaxis in bacteria or amoebae and olfactory or gustatory transduction in *Caenorhabditis elegans*[73–76]. Such relative changes of signal can be detected by one of two network motifs: the incoherent feed-forward loop (IFFL)[77,78] and the negative feedback loop with a buffering node (NFBLB)[78]. It will be important to study if any of these motifs are present in the wiring of the DPP pathway and relevant to DPP-dependent growth control. Interestingly, it seems that DPP, Yorkie and MYC could form a NFBLB[66]. Therefore, cells might adapt to relative temporal changes in DPP signalling levels through Fat signalling. Another possibility, not discussed in more detail here, is that cells could measure both C'/C and $\dot{C}/C$, or even relative temporal changes of C'/C (see Supplementary information S1 (table)), with a single sensor module[76].

Conclusions and perspectives

Recent quantifications of growth and morphogen gradient properties in the wing imaginal disc of *D. melanogaster* have allowed us to formulate and discuss current morphogenetic growth models in a slightly more precise manner, although we still cannot favour one model over another. Many components of the machinery that control morphogenetic growth in this model system are already known, but it will be interesting to identify new (or old) factors that implement this control through cell growth or cell cycle progression. However, given the wealth of qualitative information we already have in this model system, the emphasis might now be not on identifying new components in the machinery that controls morphogenetic growth, but on unravelling the dynamics of the interactions of the known components by quantitative experimental analysis.

Measurements of gradient properties so far have been either qualitative in nature or focused on average properties of cell populations. Long-term *in vivo* cultures and movies will open the way for the description and analysis of parameter distributions in individual cells. It will be interesting to measure DPP concentration and signal transduction, $\dot{C}/C$ and C'/C distributions and mechanical properties during individual cell cycles concomitantly with cell volume increase, cell cycle phase progression, growth regulatory protein levels and orientation of cell division as a function of cell position and developmental time. It is very likely that cells can measure and use a range of inputs — absolute signalling levels as well as spatial, temporal and mechanical cues — to orchestrate patterning and growth. Measuring and relating these properties to each other and to the global gradient and growth properties of the disc will hopefully allow us to complete the picture of morphogenetic growth control.

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Competing interests statement
The authors declare no competing financial interests.

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