



The wing and the eye: a parsimonious theory for scaling and growth control?

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How a developing organ grows and patterns to its final shape is an important question in developmental biology. Studies of growth and patterning in the *Drosophila* wing imaginal disc have identified a key player, the morphogen Decapentaplegic (Dpp). These studies provided insights into our understanding of growth control and scaling: expansion of the Dpp gradient correlated with the growth of the tissue. A recent report on growth of a *Drosophila* organ other than the wing, the eye imaginal disc, prompts a reconsideration of our models of growth control. Despite striking differences between the two, the Dpp gradient scales with the target tissues of both organs and the growth of both the wing and the eye is controlled by Dpp. The goal of this review is to discuss whether a parsimonious model of scaling and growth control can explain the relationship between the Dpp gradient and growth in these two different developmental systems. © 2015 Wiley Periodicals, Inc.

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INTRODUCTION

How an organ attains its final shape and size is arguably the most intriguing phenomenon in developmental biology. The question goes back to Aristotle with the concept of Entelechia,¹ the vital force that would drive the developing egg to grow until its correct final size. The concept was reformulated by Hans Driesch at the beginning of the 20th century² and is still today the subject of intense research. In the last two decades, studies of growth control have focused on signaling through morphogen gradients (reviewed in Ref 3–7). It has been tantalizing to implicate morphogens during growth control, because they mediate pattern formation (reviewed in Ref 5, 8) i.e., they tell cells their position in the developing organ so that they differentiate into the different cell

types and, at the same time, control organ size. The existence of a factor that does both things at the same time might explain how an organ arrests its growth when all the necessary cell types are in place.

Morphogens are distributed as gradients of concentration in developing tissues. It became accepted in the field that cells read the different concentrations in the gradient to obtain information about their position (reviewed in Ref 9–11). It is however less clear how a gradient of concentration can control the growth and division of the cells in the tissue. Several models have been proposed (see Figures 4 and 5): (1) as seems to be the case for morphogen-dependent establishment of pattern formation, cells could detect the concentration of one morphogen or several morphogens^{12–17} (see Figure 4(a)); (2) adjacent cells could detect the differences of morphogen concentration and stop dividing when the difference is minimal^{18,19} (see Figure 4(c) and (d)); (3) cells could measure the temporal changes of morphogen concentration²⁰ (see Figure 5); and (5) mechanical stresses in the tissue could influence the proliferation and growth of cells^{21–23} (see Figure 4(b)).

What is the relative importance of these potential mechanisms is still a matter of debate (reviewed in

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Ref 5, 7). Different scenarios of morphogen gradients might use different mechanisms of growth control. It is therefore indeed interesting to study how morphogen gradients function in different developmental setups to find whether nature found different solutions to growth control or, alternatively, the same solution is used in different organs, even if *prima facie* they appear to develop so differently.

Model systems of choice to study the function of morphogens are the fly imaginal discs, the primordia of the adult epidermal structures in *Drosophila*. In particular, much of our understanding of the function of morphogens was derived from work in the mesothoracic disc and its appendage, the wing (reviewed in Ref 5, 7, 24, 25). A recent report on morphogenetic growth control has focused on an imaginal disc whose development appears very different to the wing disc, the eye imaginal disc.²⁶ In this review, we consider and compare the two systems to revise our knowledge of growth control by morphogens. The goal is to discuss whether a parsimonious model of growth control can explain proliferation in the two systems, which appear so different. It is attractive to think that any plausible mechanism of growth control should be able to function in different systems. However, it is worth disclaiming here that, although intellectually rewarding, parsimony is in fact not necessary: in reality, different mechanisms might contribute to growth control to different extents in a particular tissue and/or they could be responsible for growth control in different organs during development.

THE WING AND THE EYE

Imaginal discs are the primordia of the epidermis in the adult fly, the epithelium that covers the animal and forms the hardened cuticular structures and appendages such as the legs, wings, antenna, eyes, genitalia, and so on.^{27,28} Imaginal discs are set apart during embryogenesis and form an epithelial sac of cells that bud out of the body wall of the worm-like larva. The imaginal cells proliferate and the disc grows during larval stages. They arrest proliferation around pupariation. In the pupa, the cells differentiate and the discs evert out of the body wall to form the different appendages with their final differentiation, shape, and size.²⁸

The mesothoracic wing disc starts out of a group of around 50 cells, which are set apart from the epidermis in the embryo.^{28,29} The mesothoracic discs give rise to the mesothorax, which is composed of the notum (the dorsal body wall in the fly) and its appendage, the wing blade.³⁰ Morphogen analysis has been focused in the wing blade (see Figure 1).

Morphogens pattern the wing with four longitudinal veins and two crossveins. Veins are thickenings of the epithelium that give robustness to the wing blade for flight. The disc starts growing during the first larval stage (instar) and goes through 10 rounds of mitosis to end up with about 50,000 cells.^{27,31} The proliferation rate slows down as the tissue grows²⁰ and proliferation ends during pupariation with two waves of mitosis from the vein primordia toward the intervein regions.³² Differentiation of the wing imaginal epithelium takes place later during pupariation and only after proliferation has arrested in the whole epithelium.^{31,33} The development of the eye appears radically different.

The eye–antennal disc gives rise to most of the head of the fly: the eye, the antenna, and the head capsule between the two.³⁴ The rest of the head is composed of a number of mouth appendages, which are derived from other, distinct imaginal disc.³¹ The compound eye of *Drosophila* is composed of around 700 ommatidia, each of which is formed by eight different photoreceptors, four non-neural cone cells, and two pigment cells.³⁴

The eye–antennal disc also starts in the embryo, but this time it initiates its development by setting aside two groups of cells from the embryo, the eye and the antenna primordia, to establish two discs that fuse into a single structure. The eye part of the disc starts out of six primordial cells,³⁵ reviewed in Ref 36. Proliferation also starts during first instar and the disc grows in size during second and third instar larval stages. Halfway through the third instar, a dorsoventral fold in the epithelium, the so called morphogenetic furrow, appears close to the posterior end of the eye disc.^{37,38} This fold separates the disc into two parts: a small posterior piece where the ommatidia start their differentiation and the rest of the disc, composed of undifferentiated, proliferating cells^{34,37,38} (see Figure 1). From this point on, the fold sweeps anteriorly as cells differentiate into ommatidia in a posterior to anterior wave. We do not know many details of the time course of differentiation of wing cells, but in any case differentiation occurs only after proliferation is arrested. In the eye disc, in contrast, differentiation occurs in a posterior to anterior wave and proliferation in the disc continues as differentiation is still taking place somewhere else in the disc.^{37,38}

Regardless of these differences, gradients of the same morphogen molecules act to control patterning and growth both in the wing and eye discs. In this review, we will focus on a particular morphogen, Decapentaplegic (Dpp), which controls growth in the wing (reviewed in Ref 7, 24) and in the eye.^{26,37,39–42}

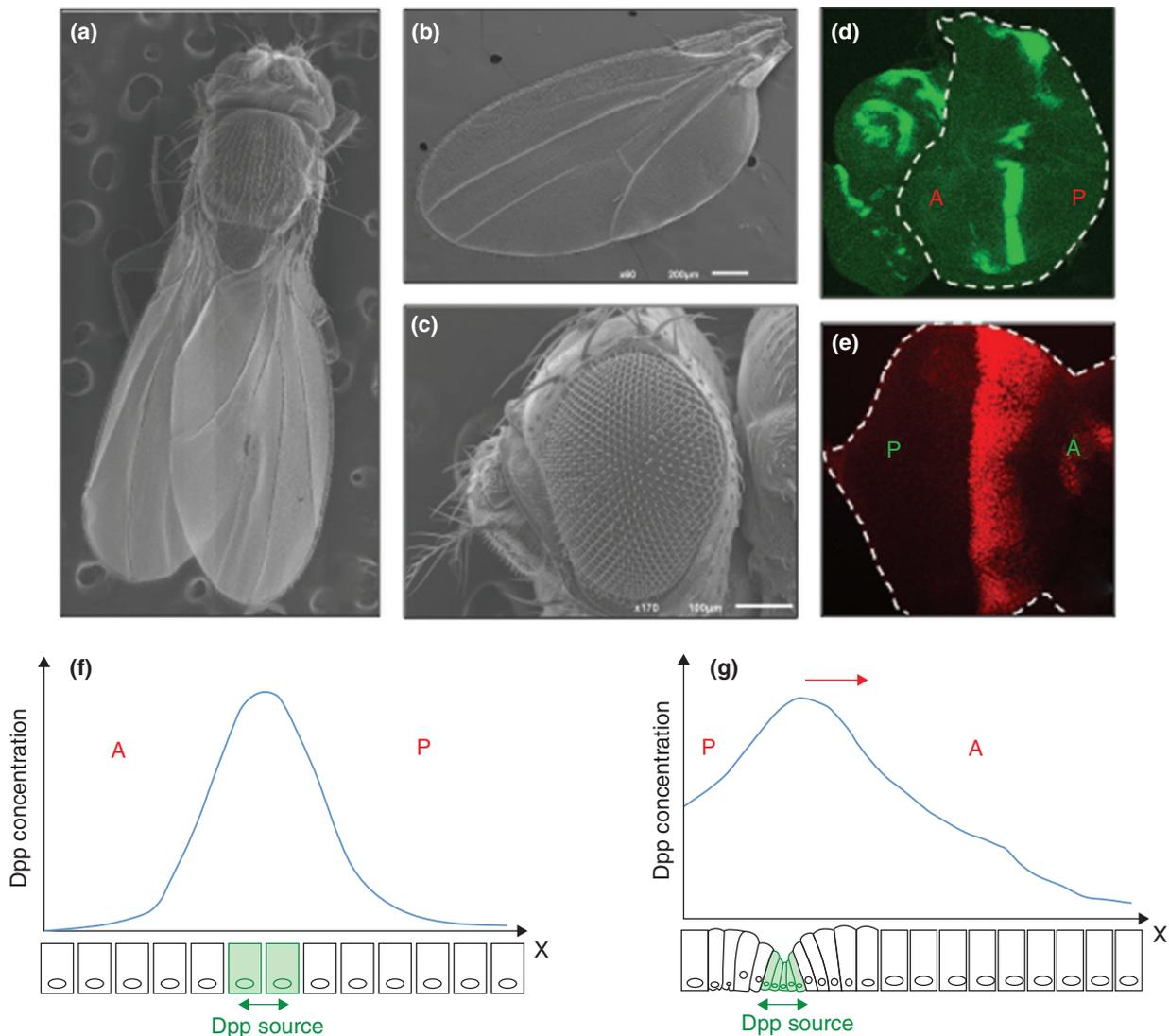


FIGURE 1 | Wing versus Eye. SEM images of the entire fly (a), the wing (b), and the eye (c). Confocal microscopy images of the wing (d, green signal is the fluorescently labeled Decapentaplegic (Dpp), the wing disc is outlined by the white punctuate line) and eye (e, the red signal is an antibody staining of Hairy, the target gene of Dpp, the eye disc is outline by the white punctuate line) discs with corresponding Dpp spatial profiles (f and g). Letters A and P indicate the anterior and the posterior compartments, respectively. The red arrow in (g) represents the direction of the movement of the furrow.

How does Dpp act in both structures? Is there a common mechanism of growth control in both systems?

MORPHOGEN GRADIENTS: DECAPENTAPLEGIC

Morphogens are secreted signaling factors, which are produced in a distinct group of cells in a developing tissue, termed the morphogen source. Morphogens spread from the source and form gradients of concentration in the target tissue consisting of cells that do not produce the morphogen. It has been suggested that the gradient of morphogen concentration

endows target cells with positional information: cells can ‘read’ the morphogen concentration and activate different target genes as a function of the concentration of the morphogen.^{9–11} Different target genes are activated above distinct concentration thresholds and therefore target genes are expressed in domains of different width (depending on the specific threshold for a particular target gene), which straddle the domain defined by the morphogen source.

Although still a matter of debate, a number of secreted signaling molecules have been proposed to work as morphogens.^{39,43–46} These include molecules of the Wnt, Hedgehog, fibroblast growth

factor (Fgf), and bone morphogenic protein (BMP) family of ligands. Dpp is a BMP superfamily member. Like other BMPs, Dpp is secreted as a homodimer, which binds a heterodimeric receptor, composed of type I [in *Drosophila* Thickveins (Tkv) or Saxophon] and type II receptors (Punt or Wishful thinking).^{47,48} Downstream the receptor, a transduction pathway involves phosphorylation of a transcription factor (the RSmad Mad),^{14,48} which in turn represses a transcriptional repressor, Brinker (Brk).^{49–52} Brk and Mad together regulate the transcription of target genes.^{50,53–55} In *Drosophila*, target genes include the anti-Smad Daughters against Dpp (Dad)⁵⁶ and the Zinc-finger transcription factors Spalt (Sal)⁵⁷ and Optomotor blind (Omb)⁵⁸ in the wing and the basic helix-loop-helix (bHLH) transcription factor Hairy in the eye,⁵⁹ among others.

Dpp is secreted from a distinct source and spreads into the tissue to be distributed as a gradient of concentration,^{13,17,60} see Figures 1 and 2. The

gradient distribution is governed by a balance of ligand diffusion and ligand clearance.⁶¹ Clearance in the imaginal discs occurs mainly by lysosomal degradation.¹³ In the embryo, a complex machinery of extracellular proteases also contributes to clearance of Dpp (reviewed in Ref 62)

The gradient profile can be characterized by two ‘descriptor’ parameters: the amplitude of the gradient, C_0 (i.e., the maximal concentration of the ligand gradient, which happens at or close to the source boundary) and its decay length, λ (i.e., the distance from the source at which the concentration decays by a fixed proportion, e.g., C_0/e , where e is the natural number).^{61,63} The shape of the morphogen gradient can be complex, but a useful description is to simplify it to an exponential profile.^{60,61} Indeed, if Dpp spreading in the tissue is dominated by its diffusion and degradation, then Dpp changes in concentration are captured by a simple differential equation, which depends on diffusion and clearance. At steady state, a

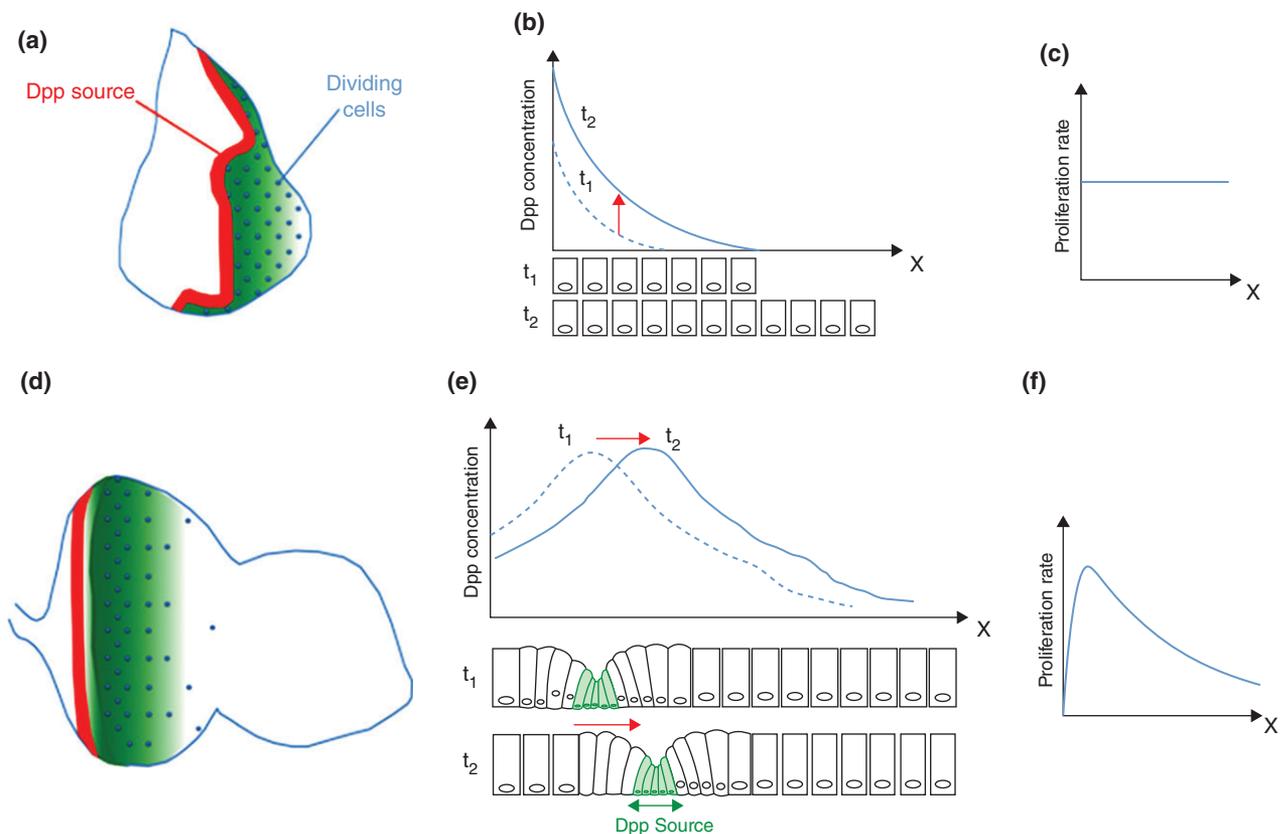


FIGURE 2 | Decapentaplegic (Dpp) gradient and proliferation profiles. (a) Dpp is secreted from a distinct source in the center of a wing disc (in red) and spreads in the target tissue to form a gradient of concentration. (b) The Dpp concentration profile in the target tissue of the growing wing disc increases in width and in amplitude from t_1 to t_2 . (c) The proliferation rate in the wing disc is roughly homogeneous in the entire target tissue. (d) In the eye disc Dpp is transcribed at the morphogenetic furrow (in red) that separate the differentiating ommatidia from the anterior undifferentiated cells (on the right of the Dpp source). (e) The furrow sweeps across the tissue from anterior to posterior. This results in a moving Dpp gradient from t_1 to t_2 . (f) As the Dpp gradient starts moving, growth in the anterior target cells becomes strongly position-dependent: there is a peak of proliferation in front of the furrow. Anterior to this peak the growth rate decays.

solution of this differential equation is

$$C(x) = C_0 e^{-x/\lambda} \quad (1)$$

where $C(x)$ is the concentration of Dpp at a distance x from the source (for a discussion of this solution see Ref 25). In this case, C_0 and λ are related to the diffusion coefficient D and the clearance rate k (and the production rate v) by

$$C_0 = \frac{v}{2k} (1 - e^{-\omega/\lambda}) \text{ and } \lambda = \sqrt{D/k} \quad (2)$$

(where ω —the width of the Dpp source, see again Ref 25 for a simplified explanation of how to derive these expressions). These effective kinetic parameters (D and k) were quantitatively determined.^{61,64} Namely, D and k were independently determined using a two-dimension fluorescence recovery after photobleaching (FRAP) assay.⁶¹

Downstream the ligand, signal transduction amplifies the signal in complex nonlinear ways. However, intermediate molecules in the transduction pathway are themselves activated (activation by their transcription, their phosphorylation, or dimerization with cofactors) in the form of spatial gradients described by values C_0 and λ that are however different to that of the ligand Dpp.²⁰ While these properties of the gradient are general and apply to both the wing and the eye, their dynamics as the tissue grows is different in the wing and the eye.

DPP IN THE WING VERSUS THE EYE

In the wing, Dpp transcription is activated in the center of the imaginal disc as a dorsoventral stripe (see Figure 2). The wing is composed of two compartments of cells related by lineage that do not mix with each other. Dpp is expressed in the anterior compartment at the anterior–posterior (A/P) boundary (see Figure 2). Expression of Dpp at the A/P boundary is triggered by another secreted ligand, Hedgehog, that is activated in the posterior compartment and activates Dpp in the adjacent cells in the anterior compartment (for details see Ref 45, 65, 66). As cells in each compartment are related by lineage, the compartment boundary remains approximately in the center of the disc and the Dpp source remains also centered and generates an approximately symmetrical gradient emanating from this central source (see Figures 1 and 2).

In the eye, Dpp is transcribed at the morphogenetic furrow that separates the differentiating ommatidia from the undifferentiated cells (see Figure 2).⁴¹ Similar to the wing, in the eye, Dpp is

activated in the furrow by Hedgehog, which itself is secreted from posterior differentiating cells.^{67–69} The furrow (and thereby the Dpp source therein) sweeps across the tissue, from posterior to anterior, because as new cells enter differentiation behind the furrow, they secrete new Hedgehog.^{70–73} As a consequence, Hedgehog activates Dpp further into the anterior region. When cells enter differentiation, their Dpp expression is silenced: the Dpp source stripe translates to a more anterior position. In summary, the Dpp source, unlike in the wing, is not static in the center of the eye, but moves from posterior to anterior as the wave of differentiation takes place.

In addition, the Dpp signaling gradient is not symmetrical in the eye (see Figure 2): Dpp signaling is activated in the anterior undifferentiated cells,⁴² where it contributes to control proliferation,^{37,39–42} but not in the posterior differentiating ommatidia, which might lack some factor in the transduction pathway. Therefore, very much unlike the wing, as the Dpp source sweeps across the eye, so does the Dpp signaling gradient in the anterior undifferentiated side (see Figure 2).

Because the properties of the Dpp gradient are so different in wing and eye, and Dpp controls growth, it is to be expected that the properties of growth in the two systems are also very different.

GROWTH IN THE WING VERSUS THE EYE

In the wing, growth is approximately homogeneous^{20,74} (the proliferation rate g of cells in the tissue does not depend on the position of cells with respect to the gradient), see Figure 2. Homogeneous growth implies that while the distance of cells (and their lineages) $x_{\text{cell}}(t)$ from the source boundary increases as the tissue grows during the developmental time t , the relative position is constant ($r_{\text{cell}}(t) = x_{\text{cell}}(t)/L(t) = \text{constant}$, where $L(t)$ is the width of the target tissue).²⁰ At the beginning of the proliferation phase, the growth rate is high and cells divide about every 4.5 h. The growth rate decreases exponentially (with a decay time of $\tau = 42$ h) until time between divisions reaches about 40 h at the end of the third instar before pupariation.²⁰

In the eye, early growth rates are also rather homogeneous (although no quantitative data are available during this early stage). However, when the Dpp gradient starts moving, halfway through the third instar, growth in the anterior target cells becomes strongly position-dependent: there is a peak of proliferation in front of the furrow (the so-called first mitotic wave)²⁶ see Figure 2. Anterior to this peak,

the growth rate decays with a characteristic decay length, λ_g . Heterogeneous growth means that, unlike the wing, the relative positions of cells are not constant. While growth is heterogeneous in the eye, it is rather isotropic.²⁶

Another difference with the wing is that the size of the target tissue does not increase monotonically until the tissue arrests: in the eye the target tissue of undifferentiated cells anterior to the furrow first expands and then shrinks until the whole tissue is differentiated.²⁶ Indeed, the size of the target tissue in the eye depends on proliferation (which makes it grow), but also on the furrow (source) movement at a constant velocity of 3 μm per hour (which makes it shrink). The size of the target tissues initially increases, but eventually shrinks as the furrow incorporates anterior cells (the furrow moves) faster than the cells can proliferate. Effectively, from 65 to 85 h after hatching, the width of the target tissue does not change, because during this time, furrow progression approximately compensates for the widening of the target tissue due to growth.²⁶ So in the eye, for many hours there is proliferation, but tissue size does not change. Before understanding how the morphogen gradients and their behaviors could explain these different growth behaviors, it is important to understand how the gradient itself changes in a proliferating tissue.

DPP GRADIENT SCALING: UPWARD AND DOWNWARD SCALING

In the wing, the Dpp gradient, as well as the Dpp signaling gradient measured by the synthetic reporter *dadnRFP*,²⁰ expands as the tissue grows: both the amplitude C_0 and the decay length λ increase. In particular, the decay length is proportional to the width of the target tissue, $\lambda(t)/L(t) = \text{constant}$.^{7,17,20,76–78} This implies that the relative concentration gradient, $C(r, t)/C_0(t)$, is invariant during development: the gradient scales with the growing tissue. As C_0 and λ depend on the production rate, the diffusion coefficient and the clearance rate (Eq. (2)), this raises a question: which of these properties changes as the tissue grows?

Estimation of these parameters by FRAP and a reporter assay showed that Dpp production rate and diffusion coefficient vary only slightly during the growth phase, whereas the clearance rate decreases substantially as $k(t) \sim 1/L(t)^2$ with the square of the increasing width of the target $L(t)$.^{20,61} Thus, changes of the Dpp clearance rate, which are mediated by lysosomal degradation,¹³ result in Dpp gradient expansion during wing growth. Scaling was also observed downstream the signal transduction pathway when

looking at the signaling gradients monitored by the activation of the target gene *dad* and other signaling readouts^{20,78,79}

The Dpp signaling gradients also scales in the eye.²⁶ Interestingly, the gradient does not only show ‘upward’ scaling (when the target tissue expands, the gradient stretches and scales accordingly) as reported before, but also ‘downward’ scaling (when the target tissue shrinks, so does the signaling gradient).²⁶ What mechanism explains the scaling of the gradients? How are individual cells in the tissue adjusting their lysosomal degradation to the overall size of the target tissue? Indeed, how do individual cells gain information about global properties of the tissue? A zoo of possible models is starting to emerge in the literature.^{20,79–82} Any plausible model of scaling has to explain not only upward, but also downward scaling in the eye. To date, four different types of mechanism have been proposed to explain scaling: expansion–repression,⁸⁰ expansion–dilution,²⁰ advection–dilution,⁸² and two opposing morphogens⁸¹ (see Figure 3).

MECHANISMS OF GRADIENT SCALING

For a morphogen gradient to scale, a cell must acquire information about the changing size of the tissue. A number of scaling mechanisms have been proposed involving a molecule X that would ‘measure’ the current size of the tissue and transfer this information to the cells building the morphogen gradient.

Two Opposing Morphogen Gradients

An example of such a scaling mechanism proposes a system involving the morphogen and a second molecule X with sources at opposite ends of the tissue, forming exponential gradients,⁸¹ see Figure 3(c). This molecule X interacts with the morphogen according to an annihilation reaction: in the region where the morphogen and X gradients overlap (the annihilation zone), X inhibits the activity of the morphogen by irreversibly binding to it. Through the process of annihilation the concentration gradient of active morphogen therefore becomes shorter leading to a shorter gradient of signaling. The larger is the annihilation zone, the shorter is the signaling gradient. As the target tissue grows, the two gradients drift further apart from each other and the annihilation zone decreases. A smaller annihilation zone leads to a larger width of the signaling gradient. The width of the signaling gradient for a specific set of conditions can approximately scale with the growing size of the tissue.⁸¹

This scaling mechanism has some limitations. With x_t corresponding to the concentration M_t of the morphogen, one can observe the variation of

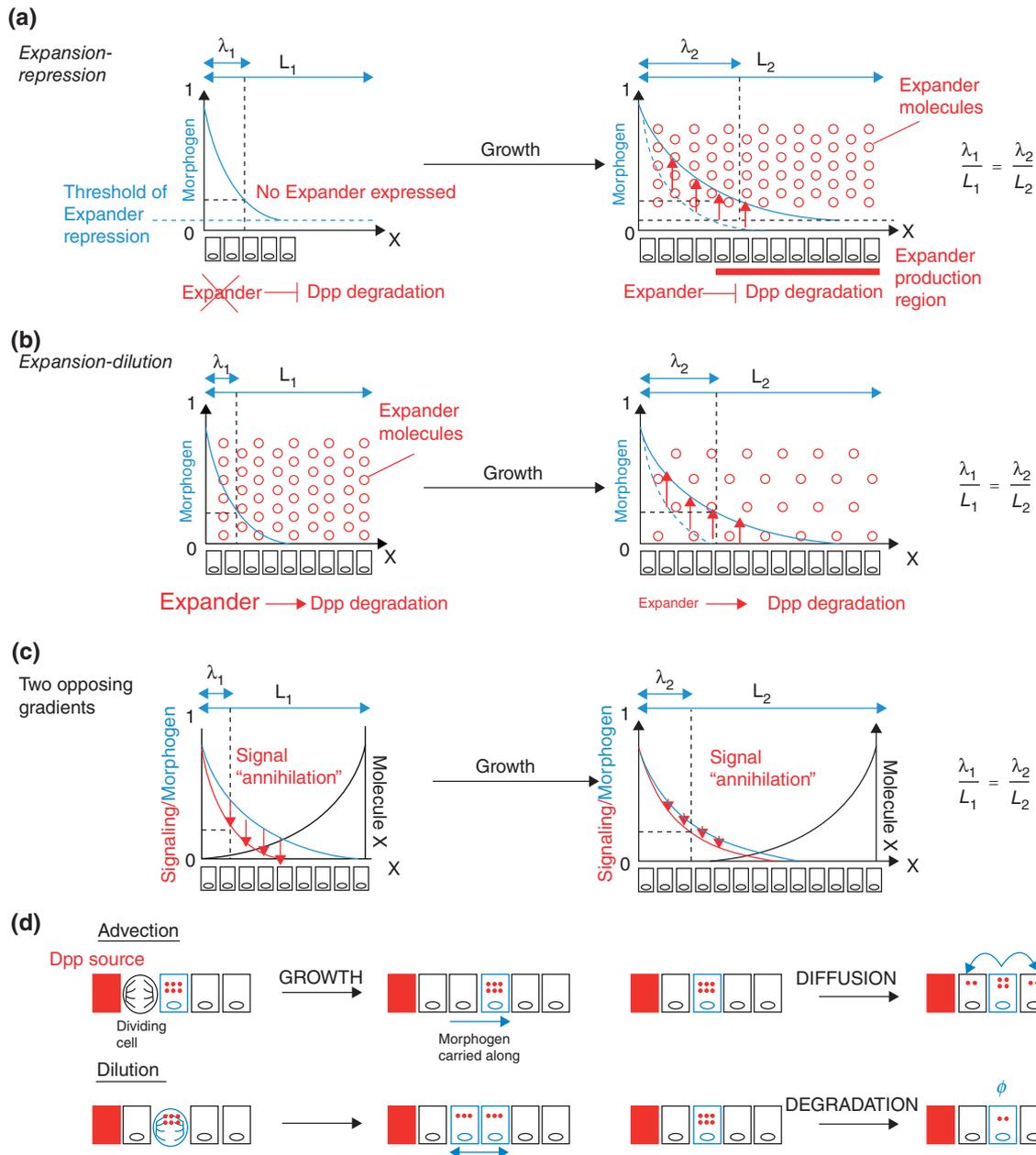


FIGURE 3 | Scaling mechanisms. (a) Expansion–repression mechanism. (b) Expansion–dilution mechanism. (c) Two opposing gradients. (d) In the case of the advection–dilution scaling mechanism, the spread of the morphogen in the target tissue is described by the kinetic parameters of morphogen transport: the diffusion and the degradation of the morphogen and by two phenomena related to the growth of the tissue: advection and dilution.

the relative position of x_t in the tissue (x_t/L) when changing the tissue size (L). Mathematically, scaling of the morphogen gradient with the tissue size occurs when the relative position of a cell corresponding to the concentration M_t does not change as the size of the tissue varies, i.e., $x_t/L \sim \text{constant}$. With scaling defined within the precision of $\delta(x_t/L) \leq 5\%$ for a given set of parameters, x_t/L plotted against L has a plateau for a restricted interval of tissue sizes. In

such a system, therefore, scaling can be provided only for small variations in system size (less than 20%). Indeed, for large tissue sizes, when the morphogen and the X gradients are too far apart, there is no overlap between the two gradients and the molecule X has no effect on the width of the morphogen gradient. On the other hand, there exists a minimal size of the tissue for which the two gradients are so close to each other that the annihilation zone extends across the entire

target tissue. In this case, the totality of the morphogen molecules are annihilated.

This limitation is crucial for the *Drosophila* wing disc, the width of which increases almost 10-fold throughout the growth phase. This scaling mechanism alone can therefore not explain scaling of morphogen gradients in *Drosophila* wing discs.

Expansion–Repression

The expansion–repression mechanism also involves a molecule X (called expander), which should convey to the morphogen gradient the information about the tissue size (see Figure 3(a)).⁸⁰ The expander molecule has a number of specific features: (1) it is highly diffusible (the lower bound for this diffusivity is $D_E \gg gL^2$ with D_E the diffusion coefficient of the expander, g the tissue growth rate and L the width of the target tissue); (2) it is long-lived relative to the tissue growth (the lower bound for expander turnover is $k_E \ll g$ with k_E , the expander degradation rate); (3) its production (i.e., its transcription plus translation) is repressed above a threshold of morphogen concentration and it works by increasing the decay length of the morphogen gradient by counteracting its degradation.

To understand how this expander works, let us think of a given target tissue, where the morphogen gradient is widely spread and represses Expander expression. As the tissue grows, the concentration of the morphogen at the edge of the tissue drops. This removes the repression of Expander expression in this region. Owing to its rapid diffusion, Expander molecules spread homogeneously in the entire tissue and widen the morphogen gradient by counteracting its degradation. This leads to an increase of the morphogen concentration at the edge of the tissue, until high morphogen levels would repress the expression of Expander.

It has been shown that during the *Drosophila* wing growth, the Dpp gradient adjusts to the size of the growing tissue by expanding^{17,20,76,78,79} and this expansion is due to the decrease of the Dpp degradation. According to the expansion–repression model, the Expander molecule somehow conveys the information about the size of the tissue to the degradation machinery of Dpp. This mechanism could therefore be at the basis of the Dpp gradient scaling in the *Drosophila* wing. Note that the expander must spread homogeneously in the tissue in order to decrease degradation uniformly. This may not be the case if growth of the tissue is fast, as for early stages of wing disc development. Expansion repression leads to imprecisions in scaling that may affect growth control.

Advection–Dilution

This model proposes mechanisms of scaling in regimes where the growth rate is fast, i.e., during early stages in wing disc development.⁸² The model emphasizes the importance of two phenomena, which become relevant when the cell cycle length is shorter than the half-life of the morphogen: advection and dilution (see Figure 3(d)). Indeed, growth of the tissue results in a movement of cells away from the source. Advection describes the movement of morphogen molecules along with these cells, to which they are reversibly associated, least to some extent. In addition, as cells divide, morphogen molecules are also diluted into the daughters. If a tissue grows homogeneously and advection and dilution phenomena are non negligible in the description of the morphogen distribution, there can be scaling of the morphogen gradient in the growing tissue without the requirement of any additional regulator of degradation. Numerical solutions of this model show that scaling over a twofold change in tissue area can be achieved if the diffusion coefficient stays within the range from 1 to about $150 \mu\text{m}^2/\text{second}$. In the case of the wing disc, measured values of the effective diffusion coefficient are much smaller and of the order $D = 0.1 \mu\text{m}^2/\text{second}$.^{20,61} It would however be consistent with the diffusion values measured by fluorescence correlation spectroscopy (FCS) in an alternative study.⁶⁴ Furthermore, in the advection–dilution scenario the value of C_0 does not increase in time as is observed in the wing imaginal disc.

Later in development, proliferation rate of cells slows down. Morphogen molecules are now degraded faster than the tissue grows, so all the growth-related phenomena affecting the distribution of the morphogen (advection and dilution) are negligible to effects of clearance of molecules. In these conditions of slow growth, the advection dilution scaling mechanism could not ensure scaling.

Expansion–Dilution

Another scaling mechanism involving an Expander molecule is the expansion–dilution mechanism²⁰ (see Figure 3(b)). According to this mechanism, Expander is initially supplied at a fix amount in each cell. It is neither produced nor degraded anymore during subsequent tissue growth. After each cell division, Expander molecules are therefore distributed between the two daughter cells and their concentration in the tissue is ‘diluted.’ In this case, Expander works by enhancing the morphogen degradation rate: $k(E) = \mu E$ with k , morphogen degradation rate; E , expander concentration; and μ a coefficient. Growth of the tissue dilutes the Expander concentration, which results in

the expansion of the morphogen gradient due to the decrease of the morphogen degradation rate in cells.

The Mechanism of Downward Scaling

The case of downward scaling of the anterior compartment of the *Drosophila* eye disc is challenging for all proposed scaling mechanisms.²⁶ As the velocity of the furrow movement becomes more important than the growth of the tissue, the anterior compartment of the eye disc becomes smaller, whereas the entire disc increases in size.

For a scaling mechanism to be able to adjust to a shrinking anterior compartment, the morphogen gradient should also shrink by increasing the morphogen degradation rates in this compartment. In Expander models (expansion–repression and expansion–dilution) for the eye disc, the expander molecules have to be confined in the target tissue, not passing the morphogenetic furrow. This is because the expander has to be sensitive to the anterior tissue size and insensitive to the size of the other part of the tissue, in which cells are already differentiating. In the expansion–repression scaling mechanism, Expander counteracts morphogen degradation. In order to increase degradation so that the gradient shortens, the amount of the expander should decrease to adjust to the reducing size of the target tissue. This conflicts with the requirement of the expander to be long-lived.

According to the expansion–dilution mechanism, Expander enhances the morphogen degradation. To adjust to the reducing size of the anterior compartment, expander would have to increase its concentration in this tissue. In a scenario in which this Expander is secreted and can be confined within the shrinking tissue, its concentration might be able to increase if the target tissue shrinks. However, the expander concentration also depends on the variation of the target tissue in the direction perpendicular to the furrow movement (L_y), which typically increases during the process. Therefore, this scenario might not be able to account for downward scaling with shrinking length L_x .

The Putative Components of the Scaling Machinery: Pentagone and Dally

While the mechanism of gradient scaling is still unclear, the molecular components that mediate scaling are beginning to be identified. We are starting to know the components, but do not yet know the underlying mechanisms. Pentagone mutants fail to correctly scale their Dpp gradient in the wing^{78,79} and in the eye²⁶: this suggests that Pentagone is part of the system responsible for scaling. Pentagone is a molecule

conserved in evolution: a Pentagone ortholog has been shown to be important during BMP signaling in zebrafish.⁸³ Pentagone has properties that make it an interesting candidate for an expander by an expansion–repression scheme⁸³: (1) it is transcribed at the edge of the Dpp gradient and its expression is repressed by Dpp signaling, (2) it is secreted, and (3) it represses Dpp clearance. Owing to the lack of available tools, there is no clear evidence in the literature yet about the distribution of endogenous Pentagone protein. It is also for the moment unclear whether Pentagone fulfills other properties of the expansion–repression model, such as its rapid diffusion and low degradation.

It is worth noting that Pentagone binds Dally, a Heparane Sulfate Proteoglycan, which uncovers a role of the extracellular matrix in the mechanism of scaling.⁸³ How the extracellular matrix could mediate scaling remains unclear, but Dally has already been implicated in the control of the Dpp spreading.⁸⁴ It will be interesting to link the proposed mechanism of scaling to the molecular and cell biological processes (extracellular matrix biology, vesicular trafficking) underlying the clearance of Dpp.

MECHANISMS OF GROWTH CONTROL BY MORPHOGENS

How can the dynamic changes of the gradient (i.e., its scaling and amplitude changes) explain growth control by morphogens? To address this question it is first necessary to understand the different models of growth control that have been proposed (see Figures 4 and 5). Three types of models can be distinguished: growth control by (1) the absolute levels of Dpp signaling (see Figure 4(a) and (b)), (2) the spatial differences of Dpp signaling in adjacent cells (see Figure 4(c) and (d)), and (3) the temporal increase of Dpp signaling (see Figure 5). These models have been described elsewhere as well as their consistency with the available experimental data (see Ref 5–7, 10, 24 for further details). Here, we will briefly review them to discuss their theoretical relevance in the context of the growth and gradient dynamics in the wing. We will then challenge these models by the recently described growth and gradient dynamics in the eye.

Absolute Levels of Dpp Signaling and Tissue Mechanics

The absolute cellular Dpp concentration C_{cell} (and Dpp signaling downstream the transduction pathway) determines the expression of target genes involved in positional information. Growth control models based

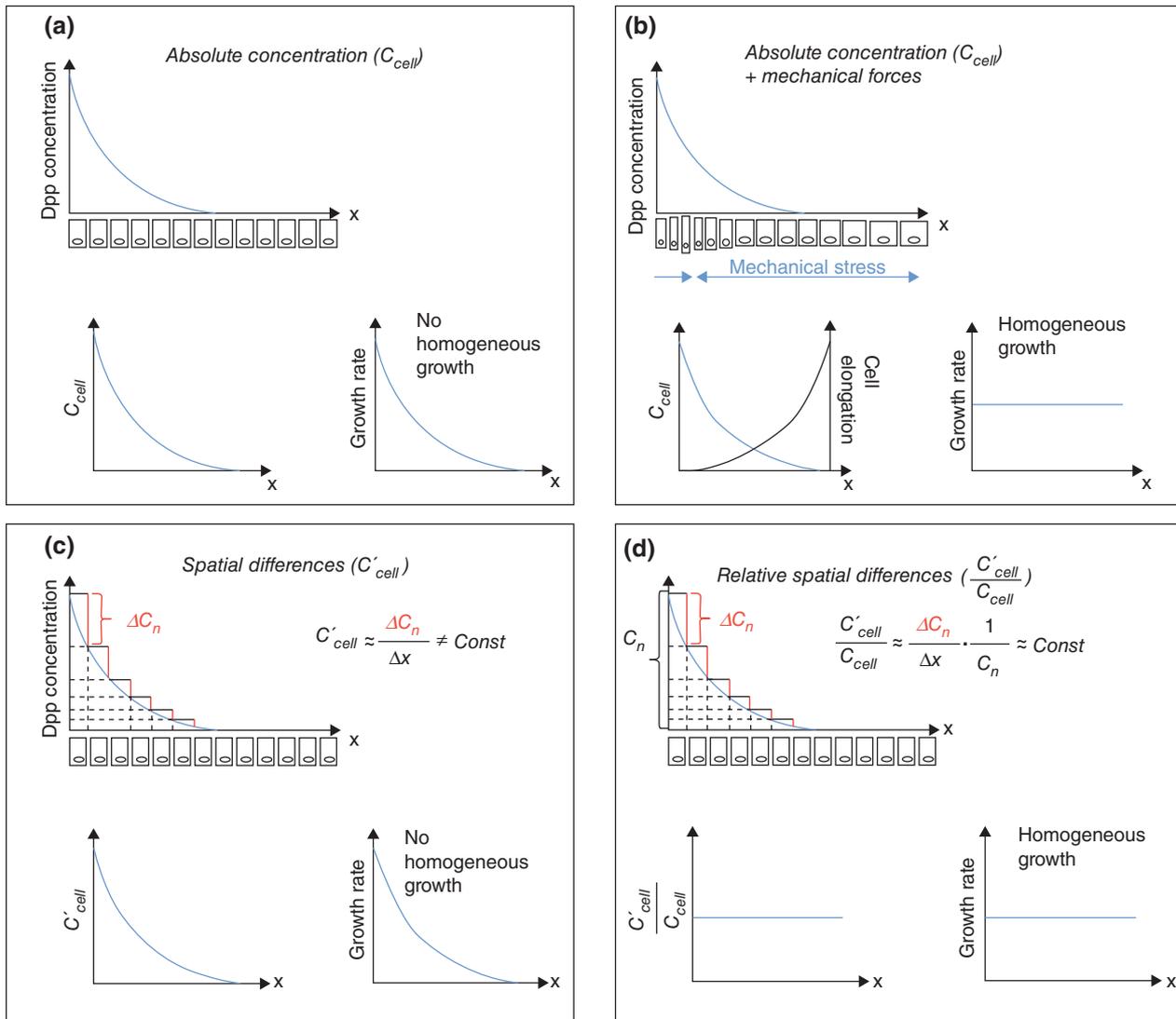


FIGURE 4 | Growth rules. (a) Absolute concentration model: the absolute concentration is not homogeneous in space thus cannot yield homogeneous growth. (b) In combination with the effect of the mechanical stress on growth, the absolute concentration model can explain homogeneous growth. (c) Spatial differences model. Spatial differences are not homogeneous in space and cannot explain homogeneous growth. (d) Relative spatial differences are spatially uniform and can explain homogeneous growth.

on the absolute levels of Dpp signaling propose that growth target genes can be activated in response to the concentration of Dpp (see Figure 4(a)).

The caveat of such models is that Dpp signaling is graded in space, but growth is homogeneous.^{20,74} To address this problem, it has been proposed that there may indeed exist a mechanical compensation of the heterogeneous Dpp signal: Dpp signaling would cause higher proliferation where Dpp concentration is higher (in the center, close to the source), but, opposing this, cells in the periphery of the gradient (lateral cells) may tend to have a higher level of proliferation, because they are exposed to stretch, whereas the central cells may be inhibited in their proliferation, because they are under compression^{21–23}

(see Figure 4(b)). Stretching and compression would influence mechanosensitive cells and might be transduced into proliferation through the Hippo pathway, which itself is sensitive to tissue mechanics^{85–87} and has been shown to be implicated in growth control.^{86–88} Peripheral stretch and medial compression would be Dpp-independent and would arise as result of the mechanical properties of a proliferating epithelium.

Higher levels of Dpp in the center increase the proliferation rate in cells which otherwise show compression-dependent lower proliferation rate. Mechanics and Dpp signaling would compensate each other and generate a homogeneous landscape of proliferation rates.^{21–23}

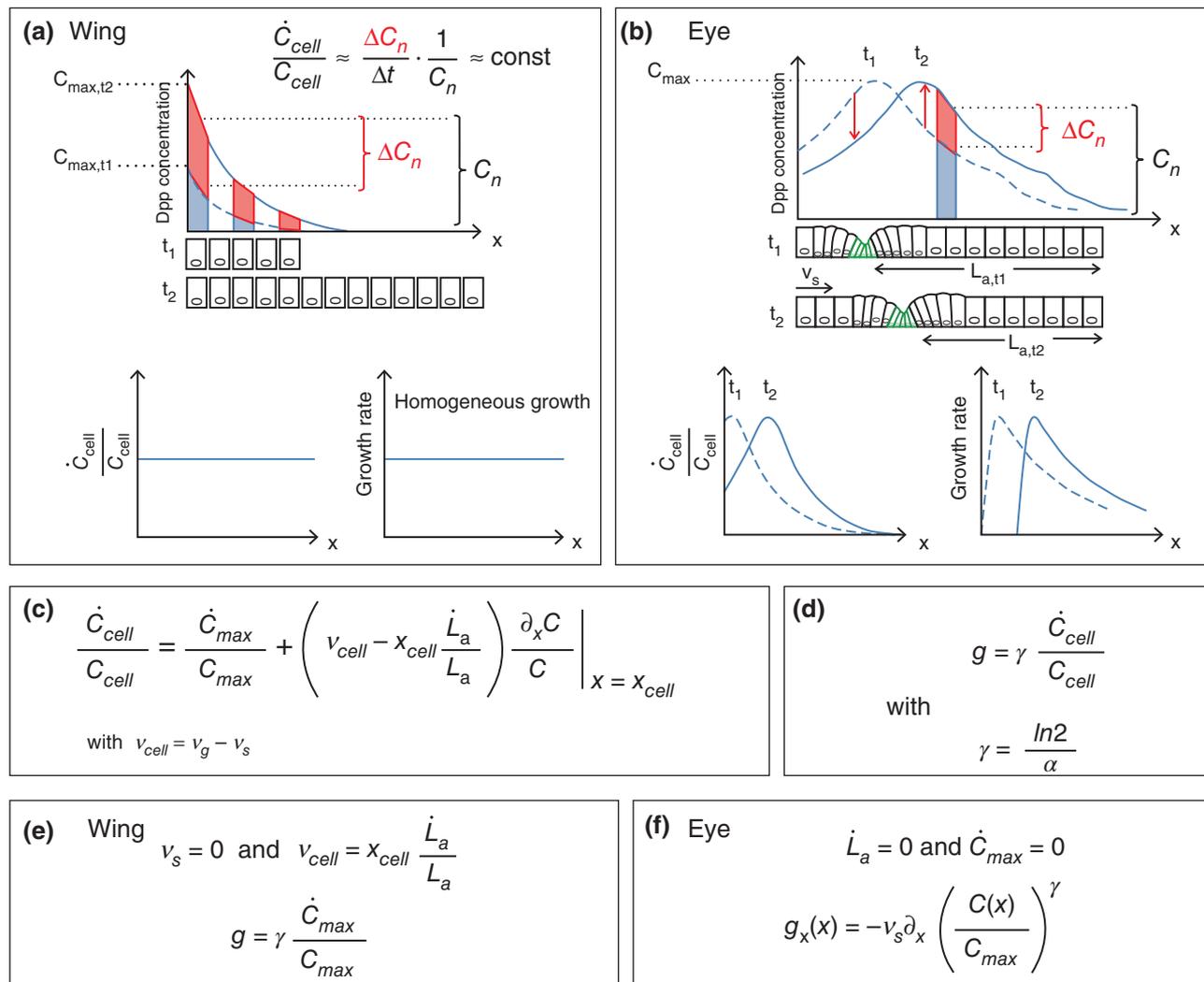


FIGURE 5 | The temporal growth rule. (a and b) Relative temporal differences (\dot{C}_{cell}/C_{cell}) can explain the uniform spatial proliferation profile in the wing disc and the spatial profile of proliferation in the eye. (c and d) General temporal growth rule expressions for \dot{C}_{cell}/C_{cell} and the growth rate (g). From these general expressions, the specific cases of the wing (e) and the eye (f) can be derived. (c) This general equation accounts for all the inputs into \dot{C}_{cell}/C_{cell} (relative changes of Dpp in time) both in the wing and in the eye: (i) The change of the amplitude C_{max} with time (\dot{C}_{max}/C_{max}); (ii) Changes due to the movement of cells relative to the Dpp source (and therefore relative to the Dpp gradient) with the velocity v_{cell} . There are two components of this movement: the movement of the source with the velocity v_s and the movement due to the proliferation of cells with the velocity v_g ; (iii) Because of the gradient scaling with L_a , the term $x_{cell} \frac{\dot{L}_a}{L_a}$ captures the change (increase or decrease) in the signaling levels of a cell, when L_a expands or shrinks, which is accompanied by an expansion or shrinkage of the gradient. (e) The temporal growth rule expression for the case of a system with a nonmoving Dpp source ($v_s = 0$) and homogeneous growth. Therefore, $v_{cell} = x_{cell} \frac{\dot{L}_a}{L_a}$ (Drosophila wing disc). (f) The temporal growth rule expression for the case in which the source moves ($v_s \neq 0$) and the target tissue length (L_a) and the gradient amplitude (C_{max}) are constant in time. The sole cause of increase of cellular Dpp concentration is therefore the movement of cells upward the gradient due to the movement of the furrow (Drosophila eye disc during the developmental time between 65 and 85 h after hatching).

Spatial Differences of Dpp Signaling in Adjacent Cells

This model suggests that the proliferation rate depends on the differences of Dpp signaling between neighboring cells and thus on the spatial slope C'_{cell} (the spatial time derivative) of the Dpp profile (see Figure 4(c)). As the tissue grows, the profile may be stretched. This

could indeed be caused by gradient scaling and would lead to a decreasing slope and thus decreasing growth rate. When a small slope, below a threshold value, is reached, then proliferation may stop.^{18,19} This model requires that cells can determine the spatial derivative of Dpp concentration (the slope of the gradient) across a cell, C'_{cell} , instead of the absolute concentration,

C_{cell} . To explain homogeneous proliferation, such a model would work in the case of a linear concentration profile, where all the cells perceive the same gradient slope ($C'_{\text{cell}} = \text{constant}$). However, as the observed Dpp profiles are typically steeper close to the source, while being almost flat in lateral cells, such models cannot account for homogeneous growth (see Figure 4(c)).

A variation of this model is that cells compute the relative spatial derivative ($C'_{\text{cell}}/C_{\text{cell}}$, the percentage of variation between cells),²⁰ see Figure 4(d). If the gradient is exponential, the percentage difference between adjacent cells in the proximo-distal axis of the gradient is approximately constant and is related to the inverse of the decay length by $C'_{\text{cell}}/C_{\text{cell}} = -1/\lambda$. Such a dependence on relative spatial derivatives could therefore account for homogenous growth. In a scaling gradient where the decay length increases and stays proportional to the target tissue width, $C'_{\text{cell}}/C_{\text{cell}}$ does decrease and could in principle cause the decrease of the growth rate until proliferation arrests below a fixed $C'_{\text{cell}}/C_{\text{cell}}$ threshold.

Changes of Dpp Signaling Over Time:

$\dot{C}_{\text{cell}}/C_{\text{cell}}$

This model suggests that relative rates of increase of cellular Dpp signaling controls cell growth and proliferation (see Figure 5). Measurement of the clearance rate of Dpp in the growing wing shows that scaling of the gradient is due to a drop of the clearance rate k of Dpp according to the relation $k \sim 1/L^2$.²⁰ This causes the decay length of the gradient to be proportional to the width of the wing tissue, $\lambda/L = 0.11$. It also has consequences for the amplitude C_0 , which is then related to the area A of the target tissue by a power-law, $C_0 \sim A^\beta$, with an exponent $\beta = \alpha/\ln 2$. Because, the gradient scales, the temporal variation of the Dpp concentration can be defined in the case of homogeneous growth (the wing disc) by the expression $C_{\text{cell}} = \dot{C}_0 f(x_{\text{cell}}/L)$, where f describes the shape of the cellular Dpp profile C_{cell} , which, because of scaling, only depends on the relative position x_{cell}/L . From this relation, it follows that the relative rate of change of Dpp levels is spatially homogenous and independent on the shape of the profile: $\dot{C}_{\text{cell}}/C_{\text{cell}} = \dot{C}_0/C_0$ (an exponential profile assumption is therefore not necessary²⁰). Because of scaling, the concentration of Dpp in a given cell C_{cell} is proportional to the concentration at the source boundary C_0 , i.e., $C_0 \sim C_{\text{cell}}$. Therefore $C_0 \sim C_{\text{cell}} \sim A^{\alpha/\ln 2}$ and thus

$$\dot{A}/A = \left(\frac{\ln 2}{\alpha}\right) \dot{C}_{\text{cell}}/C_{\text{cell}} \quad (3)$$

The implication of the relationship between the relative rate of change of Dpp in cells and the area growth rate \dot{A}/A of the target tissue is that cells divide on average when the Dpp concentration increases by a percentage $\alpha = 50\%$ since the beginning of the cell cycle,²⁰ see Figure 5(a), (d), and (e). Moreover, the growth rate i.e., the relative area variation with time ($g_{\text{cell}} = \dot{A}/A$), in the disc is proportional to the relative time derivative (i.e., $\dot{C}_{\text{cell}}/C_{\text{cell}}$) of Dpp concentration: $g_{\text{cell}} = (\ln 2/\alpha) (\dot{C}_{\text{cell}}/C_{\text{cell}})$. $C_{\text{cell}}/C_{\text{cell}}$ is homogeneous in space, therefore g_{cell} is also constant in space: growth is homogenous. This model can thus explain homogenous growth in the wing.

This correlation between growth and $\dot{C}_{\text{cell}}/C_{\text{cell}}$ is not only true in wild-type wings, but holds in other tissues, such as the halter, and in a number of mutant scenarios in which the dynamics of Dpp and growth are affected.²⁰ In order to go beyond the simple correlation relationship between the gradient changes and the proliferation, the time derivative of Dpp signaling was exogenously manipulated by using a drug-dependent expression system.²⁰ In this scenario, scaling up of the gradient in a growing tissue is driving the increase of Dpp concentration: gradient scaling ensures positive values for $\dot{C}_{\text{cell}}/C_{\text{cell}}$ and therefore growth.

It is interesting to note that the temporal model implies a mechanism at the single cell level: a single cell could determine the increase in concentration during the cell cycle and divide when it increases above $\alpha = 50\%$, or cells could compute the time derivative of signaling \dot{C}/C and convert it into a growth rate. This is a mechanism at the single cell level. Indeed, an interesting future experiment would be the live imaging of single cells while monitoring the signaling level and its time derivative.

Growth Control by Relative Rates of Change of Dpp Levels in the Eye

Would this mechanism of growth control also work in the eye? In the eye, the length of the target tissue remains roughly constant during a long phase of the larval development (65–85 h after hatching) due to the balance between proliferation of the target and the progression of the source at the morphogenetic furrow.²⁶ Therefore, during this growth phase scaling could not explain cellular increases in Dpp signaling. In addition, in a later phase the length of the target tissue decreases and therefore, there is not scaling by gradient expansion, but rather by gradient retraction.

As the morphogenetic furrow advances, the Dpp profile is carried with the moving furrow where the Dpp source is located. This implies that the concentration of Dpp in a target cell would increase

as the cell approaches the furrow and moves into the gradient.²⁶ Let us consider for simplicity the phase from 65 to 85 h after hatching formation in which the target tissue length is approximately constant (see Figure 5(b) and (f)). During this time, the only cause of increases of cellular Dpp concentration is the movement of cells toward the furrow as the furrow progresses anteriorly, i.e., cells are effectively moving upward the gradient. In this phase, if the C/C model applies (i.e., if it is true that $g_{\text{cell}} = (\ln 2/\alpha) (\dot{C}_{\text{cell}}/C_{\text{cell}})$), then the proliferation rate g_x would be related to the concentration profile of Dpp as

$$g_x = v_s \partial_x \left(\frac{C(x)}{C_{\text{max}}} \right)^{\ln 2/\alpha} \quad (4)$$

where v_s is the velocity of the furrow and ∂_x denotes the space derivative (i.e., the slope) of the Dpp profile normalized to its maximal concentration,²⁶ see Figure 5(b) and (f). Note that in this case g_x is not homogeneous in space, but depends on the shape (the slope) of the Dpp profile which depends on the position x of cells: cells move up the gradient, but the gradient has a steeper slope close to the source than far away from it (see Figure 5(b)). The model captured by Eq. (4) thus predicts a proliferation wave (a position-dependent growth rate) in front of the moving furrow.

Indeed, experimental determination of the proliferation rate showed that the precise shape of the proliferation profile (which is not homogenous, but in a wave) can be captured by this Eq. (4), which depends on the Dpp gradient profile and the velocity of the furrow. Indeed it does predict the position of the mitotic wave and the actual values of the proliferation rate as a function of position.²⁶ We estimate values of α similar to the wing: in the eye, cells would divide when the Dpp concentration increases by about 60% in the course of interphase (versus 50% for the wing).

In the phase from 65 to 85 hours after hatching in which the target tissue length is approximately constant, the proliferation rate profile depends on the velocity of the furrow and the shape of the gradient (Fig. 5F, equation 4). Consequently, in mutants that affect the gradient shape or the velocity of the furrow, the proliferation profiles could be predicted by the equation 4.²⁶ For instance, if the furrow is slowed down or stopped, the proliferation rate drops to a low, basal level, and growth becomes homogeneous as described by Eq. (4). The temporal model therefore explains, in a parsimonious way (it applies also to the wing (Fig. 5E) and the halter), in a quantitative manner, how the Dpp gradient could control growth both in the wing and the eye. It is worth emphasizing

that analysis in the phases of eye development in which there is upward and downward scaling (i.e., beyond the stages from 65 to 85 h after hatching) confirmed that this model still holds.²⁶ Moreover, a general expression, accounting for all the possible inputs into $\dot{C}_{\text{cell}}/C_{\text{cell}}$ has been proposed (see Fig. 5C for detail).²⁶ From this general expression for $\dot{C}_{\text{cell}}/C_{\text{cell}}$ and the temporal growth rule expression correlating the growth rate and the relative changes of the cellular signalling levels, the specific cases for the wing (Fig. 5E) and the eye (Fig. 5F, equation 4) can be derived.

THE EYE UNCOVERS CAVEATS FOR OTHER GROWTH MODELS

Experiments in the eye showed that the temporal model accounts parsimoniously for growth in wing and eye. What about the other models?

Absolute Dpp Signaling Levels

This model appears the least plausible by itself, although, in the wing, in combination with tissue mechanics it might still be able to account for tissue growth. However, the eye scenario seriously challenges this model. Indeed, the analysis of growth in the eye shows that the velocity of the furrow depends crucially on the movement of the furrow: it was shown experimentally by using mutants in the Hedgehog pathway, which is responsible for progression of the furrow, that if the furrow does not move, growth becomes homogeneous. In these mutants, the actual profile of Dpp is not different to the one in the wild-type eye. The actual levels of Dpp signaling in cells cannot explain the proliferation rates in the cell.

In addition, in other experiments in which Dpp is ubiquitously expressed in all the cells, rather than higher, the proliferation rates are lower in the eye²⁶: the levels of signaling and proliferation rates are anticorrelated in this scenario.

The Slope Model

The slope model remains a plausible model in the wing (see also Ref 7 for a discussion of this): in a scaling gradient, there is correlation between the relative slope $\dot{C}'_{\text{cell}}/C_{\text{cell}}$ and the growth rate. Also, while the threshold value of the slope at which different wing mutant conditions or the halterium arrest proliferation varies depending on the experiment, this might in principle be due to unknown particularities of these tissues. The control parameters might not be universal (not parsimonious), but the model could account for homogeneous growth.

In the eye, it becomes clear that the slope model cannot account for the observed growth patterns. In the slope model, the furrow velocity should be irrelevant for growth, unlike what is observed in the Hedgehog mutants.²⁶ In these experiments, the steepness of the gradient is close to those in wild-type discs.

Mechanical Stress

As the furrow moves, cells do change their shapes as they move into the furrow⁸⁹: furrow movement could in principle cause a heterogeneous pattern of mechanical stress which might correlate with the mitotic wave and the profile of proliferation in the eye. However in mutants that affect the shape of the Dpp signaling profile (ubiquitous Dpp signaling or nonscaling *pentagone* mutants), the velocity of the furrow is not affected.²⁶ In this situation, the potential landscape of mechanical stress would be like that present in wild type. However, in these mutants, the proliferation profile is dramatically affected.²⁶

Indeed, it is not trivial to find a model that can explain the proliferation landscape of mutants in which Dpp is ubiquitously expressed. In the wing, it causes higher proliferation far from the source.²⁰ In the eye, the effect is the opposite: lower proliferation far from the source.²⁶ This mutant, in which Dpp is ubiquitously expressed, does support the temporal model because the different profiles of proliferation in wing and eye are predicted quantitatively by a single equation: $g_{\text{cell}} = (\ln 2/\alpha) (\dot{C}_{\text{cell}}/C_{\text{cell}})$.^{20,26}

GROWTH IN THE ABSENCE OF THE DPP SIGNALING INPUT

The temporal model explains in a quantitative manner, how Dpp signaling could control growth in two different developmental contexts: the wing and the eye. What happens to Dpp signaling and tissue growth in a situation with no Dpp input?

The Dpp-Independent Growth in the Wing

Dpp signaling can be activated in two ways downstream of Dpp: direct activation by the transcription factor Mad and activation by Mad through repression of the transcriptional repressor Brk.⁹⁰ In the wing disc, in *brk dpp* double mutants²⁰ and in *brk mad*^{91,92} mutant clones, cells could still proliferate.

To account for growth in the absence of the Dpp input, a model has been proposed in which the key role in growth control is attributed to the inhibitor of cell proliferation Brk^{74,91} (reviewed in Ref 5). The

Dpp contribution to growth control in this model is exclusively by shaping the profile of *brk* expression to yield uniform growth. This model is based on the hypothesis that a residual proliferation pattern exists, which is Dpp-independent. This model could therefore explain cell proliferation in *brk dpp* double mutants²⁰ and in *brk mad*^{91,92} mutant clones: cells do not require Dpp to proliferate in general, Dpp is rather important for cells to proliferate homogeneously in space. What are the signals that directly control growth, yielding the residual proliferation pattern of this model remains to be uncovered.

A number of recent findings challenge this model. Increase of Dpp signaling in medial regions of the disc,¹⁹ where *brk* expression is very low in general, would not affect cell proliferation in these regions. Nevertheless, overexpression of a constitutively active form of Tkv (Tkv^{QD}) in medial cells causes overproliferation of cells.¹⁹ Moreover, it has been shown that both in *brk dpp* double mutants²⁰ and in *brk mad*^{91,92} mutant clones, the levels of the dad-nRFP reporter was significantly reduced but not absent.⁹² Analysis of the dad-nRFP signaling profile in these conditions revealed that it is graded in space and that it changes in time.⁹² These changes of dad-nRFP are Dpp-independent: they do not reflect Dpp signaling, but the dynamic behavior of genes that can be under the control of Dpp.

A tempting explanation of this observation is that signaling, measured by the dad-nRFP reporter, controls proliferation through the temporal rule. Indeed, even though Dpp represents an important input into this growth-controlling signal, it may nevertheless not be exclusive. Thus, in the absence of Dpp, the graded pattern and the temporal changes of this signal might still be generated through some yet unknown Dpp-independent inputs.

The Dpp-Independent Growth in the Eye

The study in the eye of growth and Dpp signaling in the absence of the Dpp input²⁶ made an important contribution to understanding Dpp-independent growth. As previously mentioned, in the eye, the shape of the proliferation profile depends on the signaling profile and the velocity of the furrow. Experiments affecting the furrow velocity in mutant conditions where the Dpp input into signaling is removed (i.e., *mad brk* mutant clones) showed that in the absence of Dpp, the proliferation profile is still dependent on the velocity of the furrow and can still be predicted quantitatively by the Hairy profile. As in the wing, in the eye disc in the absence of Dpp, a Dpp-independent signal can drive growth according to the temporal rule.²⁶

CONCLUDING REMARKS

The *Drosophila* wing and eye are two very different developmental systems to study scaling and growth: if the wing is a system with a static morphogenetic source and homogeneous growth,^{20,74} the eye has a moving source^{67–69} and a graded proliferation profile.²⁶ In the wing, the Dpp signaling gradient scales with the growing tissue,^{7,17,20,76–78} whereas the Dpp signaling gradient in the eye scales in both directions: upward and downward.²⁶ The goal of this review is to discuss whether a parsimonious model of scaling and growth control can explain the relationship between the Dpp gradient and the growth in these two different developmental systems.

Some indications of a common scaling mechanism come from recent studies on the roles of Pentagone and Dally in Dpp scaling in the wing and the eye.^{26,78,79} These proteins could be important for future research to dissect the Dpp gradient scaling at the subcellular level. Moreover, discovery of the bidirectional Dpp signaling scaling in the eye uncovers difficulties for currently proposed scaling models. Such models have to explain not only the upward, but also the downward scaling in the eye.

The situation is more promising in the search of a conserved mechanism controlling growth: the recently proposed temporal rule is at the moment the only model that can correlate the proliferation and the Dpp signaling profiles in both the wing²⁰ and the eye.²⁶ Moreover, the temporal rule appears to be even more general, as in the absence of Dpp, the growth profiles in both systems can be correctly predicted by the corresponding spatial profiles of the Dpp target genes.^{20,26,92}

It is not surprising that in such an important phenomenon as growth control, several redundant inputs could ensure a reliable control. Indeed, it has previously been shown that there are Dpp-independent inputs into dad and Hairy expression,^{39,53,91,93} It is nevertheless interesting that, both in the wing and the eye, growth appears to be controlled by the temporal rule through Dpp-dependent and, in the absence of the latter, Dpp-independent signaling.

As previously mentioned, the temporal model implies a mechanism at the single cell level: a single cell can compute the increase in concentration during the cell cycle and divide when it increases above 50%. Experiments at the single cell level form an important future direction in understanding growth control by morphogen gradients.

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