

Model for the regulation of size in the wing imaginal disc of *Drosophila*.

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For animal development it is necessary that organs stop growing after they reach a certain size. However, it is still largely unknown how this termination of growth is regulated. The wing imaginal disc of *Drosophila* serves as a commonly used model system to study the regulation of growth. Paradoxically, it has been observed that growth occurs uniformly throughout the disc, even though Decapentaplegic (Dpp), a key inducer of growth, forms a gradient. Here, we present a model for the control of growth in the wing imaginal disc, which can account for the uniform occurrence and termination of growth. A central feature of the model is that net growth is not only regulated by growth factors, but by mechanical forces as well. According to the model, growth factors like Dpp induce growth in the center of the disc, which subsequently causes a tangential stretching of surrounding peripheral regions. Above a certain threshold, this stretching stimulates growth in these peripheral regions. Since the stretching is not completely compensated for by the induced growth, the peripheral regions will compress the center of the disc, leading to an inhibition of growth in the center. The larger the disc, the stronger this compression becomes and hence the stronger the inhibiting effect. Growth ceases when the growth factors can no longer overcome this inhibition. With numerical simulations we show that the model indeed yields uniform growth. Furthermore, the model can also account for other experimental data on growth in the wing disc.

KEY WORDS: development, *Drosophila*, growth control, Decapentaplegic, computer simulations

INTRODUCTION

During development it is crucial that growth ceases when tissues or organs have attained a certain form and size. However, the regulation of final tissue size is poorly understood. The wing imaginal disc of *Drosophila* is often used to study the regulation of growth. A wealth of data is therefore available on growth and development of this tissue, and models that can account for all of this data likely represent good descriptions of the biological situation.

Drosophila imaginal discs are simple epithelial structures that give rise to the adult body structures. The wing disc contains about 30 cells at the beginning of the first instar larva and reaches at metamorphosis, almost 4 days later, a number of about 50,000 cells (Milan et al., 1996). The adult wing is produced by the eversion of the wing disc and its cells neither divide nor grow. The size of the adult wing is therefore predetermined by the final size of the wing disc (Day and Lawrence, 2000).

Decapentaplegic (Dpp) plays an important role in regulating growth in the wing disc. In *dpp* mutants the wings are reduced to small stumps whereas overexpression of *dpp* leads to larger wing discs (Burke and Basler, 1996; Capdevila and Guerrero, 1994; Lecuit et al., 1996; Posakony et al., 1990). *dpp* is expressed in a narrow stripe

of anterior cells adjacent to the anteroposterior compartment boundary (Basler and Struhl, 1994; Posakony et al., 1990; Tabata and Kornberg, 1994) and forms a gradient in anterior and posterior directions (Entchev et al., 2000; Teleman and Cohen, 2000) (Fig. 1). Because of the growth promoting effect of Dpp, it may be expected that growth preferentially occurs where Dpp activity is highest. However, as judged by the occurrence of cell proliferation, this is not the case and growth occurs uniformly throughout the disc (Milan et al., 1996).

Several models have been formulated for the regulation of size (Day and Lawrence, 2000; Garcia-Bellido and Garcia-Bellido, 1998; Nijhout, 2003). To our knowledge, the gradient model of Lawrence and Day is the only model that explicitly takes into account a role for a centrally produced growth factor in the wing disc (Day and Lawrence, 2000). In its simplest form, the model proposes that the high Dpp level in the center and the low Dpp levels at the ends of the disc are fixed. Growth anywhere in the disc extends the gradient and thus reduces its rake. Cells only grow when the local Dpp gradient is sufficiently steep and therefore cell proliferation ceases when the local steepness falls below a threshold (Day and Lawrence, 2000). This model predicts that growth does not occur in a disc with homogeneous Dpp signaling, since the Dpp slope is near zero in such discs. However, considerable growth has been observed in wing discs with homogeneous Dpp signaling (Martin-Castellanos and Edgar, 2002; Nellen et al., 1996), thus contradicting the gradient model in the case of the wing disc. There are several other models available to account for uniform growth in the presence of a Dpp gradient (Gibson et al., 2002; Rogulja and Irvine, 2005; Serrano and O'Farrell, 1997; Shraiman, 2005), but none of them explicitly considers final disc size as well.

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Here we formulate a new model for the regulation of size in the wing imaginal disc, which can simultaneously account for the observed homogeneous growth in the presence of a Dpp gradient as well as alterations of growth caused by experimental interventions.

RESULTS

The model

There are a number of biological assumptions underlying the model. Firstly and most fundamentally, it is assumed that growth is not solely regulated by growth factors, such as Dpp, but by mechanical forces as well. In particular, it is posited that compression within the plane of the wing disc inhibits net growth and that stretching stimulates it. A second important assumption constitutes the presence of another growth factor, which forms an activity gradient perpendicular to that of Dpp, i.e. its activity is highest at the dorsoventral boundary (Fig. 1). Growth is induced if both Dpp and the second growth factor are present. This implies that the net growth factor activity is highest in the center of the wing disc and lowest in the peripheral regions. Thirdly, it is assumed that the Dpp activity gradient and that of the other growth factor are scaled, i.e. that they adjust to changes in wing disc size during growth. Fourthly, the model assumes that there is no growth when growth factor levels as well as stretching are too low. Lastly, stretching is assumed to only induce growth above a certain threshold. For the model as presented below, this is equivalent to a stimulation of growth above a certain cell elongation threshold.

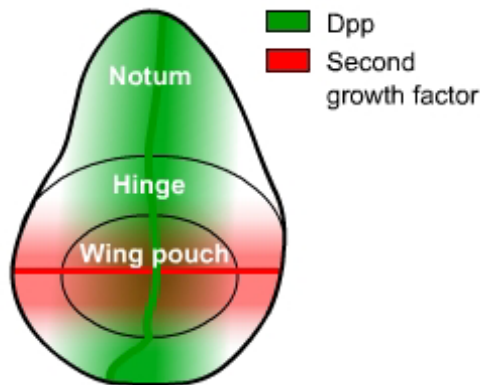


Fig. 1. Different regions of the wing imaginal disc. Dpp is produced in a stripe adjacent to the anteroposterior boundary and forms a gradient. According to the model, a second growth factor gradient is formed perpendicular to the Dpp gradient and the presence of both growth factors is required to induce growth. Then, the distribution of net growth factor activity resembles a tent, with highest activity in the center of the disc and lowest activity at the edges. Our model does not include growth in the notum.

Qualitatively, the model can be described as follows: At a very early stage of wing disc development, Dpp and the second growth factor are present, but mechanical stress has not yet evolved. In the center, growth will be induced by

the combined high activity of Dpp and the second growth factor. In contrast, growth will not be induced in the more peripheral regions, because of lack of stimulation by either growth factors or mechanical forces (Fig. 3A,B). Growth in the central part naturally results in stretching of the peripheral regions (Fig. 3B). Since it is assumed that stretching has a growth promoting effect, growth will then also be induced in the peripheral regions. However, growth is only induced above a certain threshold of stretching, such that the peripheral regions remain stretched to some extent. This stretching of the peripheral regions compresses the center, thus exerting an inhibitory effect on this region. As the disc grows, the peripheral regions become wider, such that they cause an increased compression of the center. Growth ceases in the center when the positive effect on growth exerted by the growth factors is completely counteracted by the negative effect exerted by compression. When growth ceases in the central region, the peripheral parts do not get stretched sufficiently any more for the induction of growth. Therefore, growth in the peripheral regions automatically ceases as well.

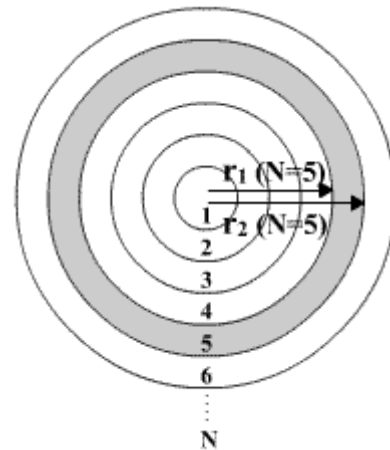


Fig. 2. Subdivision of the disc in the simulation. The disc is subdivided into N small concentric rings, with an inner radius r_1 , and an outer radius r_2 .

Simulation results

In order to assess whether the qualitative model described above can in principle account for the experimentally observed homogeneous growth and to get more insight into other features of the behavior of the model, we formulated a quantitative version of the model and numerically simulated it. This quantitative model, including additional assumptions that were made in order to facilitate the modeling, is described in detail in the materials and methods section. Here, the simulation results will be discussed. Fig. 4 shows different time points of a simulation experiment.

At the start of the simulation, growth only occurs in the center of the disc (Fig. 4A). This growth stretches the directly adjacent regions (Fig. 4B), causing them to grow (Fig. 4C). The stretching then spreads to the most peripheral parts, such that growth is induced in these

regions as well (Fig. 4C,D). This results in homogeneous growth throughout the disc at a time point when the disc still has a very small size (Fig. 4D). During the whole growth process, the peripheral region stays slightly stretched, thus compressing the center (Fig. 4D,E). In late discs, this stretching is more pronounced in the regions adjacent to the center than in the most peripheral regions (Fig. 4E,F). Stretching throughout the peripheral region contributes to compressing the center, decreasing its growth rate. Consequently, the growth rate in the peripheral regions decreases as well, causing growth to stay uniform throughout the disc (Fig. 4E). Eventually, growth ceases completely when the disc has a radius of 126 cells, corresponding to about 50,000 cells (Fig. 4F).

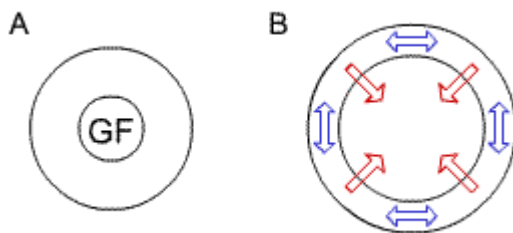


Fig. 3. Principle of the model. Initially growth occurs in the center where the growth factor concentration is high (GF in A). This growth causes the peripheral regions to stretch and the center to be compressed (B). The stretching in the peripheral regions induces growth there. Even though this growth reduces the stretching in the peripheral regions, some stretching remains. As a consequence, the center will still be compressed to some extent, which inhibits growth in this region. The wider the peripheral regions, the larger the compression becomes. Finally, growth stops when the inhibiting effect exhibited by compression compensates for the growth promoting effect of growth factors in the center.

The simulation experiment shown in Fig. 4 thus shows uniform growth shortly after growth has started and growth stays uniform until the disc reaches its final size. This can be explained by considering that stretching increases in the surrounding regions as long as the center grows faster than these regions. Increased stretching in turn leads to increased growth. Stretching and growth rate will thus increase in the surrounding regions until there is no difference in growth rate present anymore. We therefore conclude that the model is consistent with the experimentally observed homogeneous growth, since it naturally yields uniform growth as long as the growth factor concentration is higher in the center than in the peripheral regions.

The inherent tendency of the system to yield uniform growth also suggests an explanation as to why regions adjacent to the center become more stretched than the most peripheral regions. The less peripheral regions are compressed to some extent by the most peripheral regions, leading to a temporal decrease of growth. This decrease in growth leads to an increase in stretching until the growth disadvantage is completely compensated for and growth is uniform. The most peripheral regions do not have this

growth disadvantage and therefore they are not additionally stretched.

Evaluation of the model with available experimental results

Since the wing imaginal disc serves as a model system to study the regulation of growth, a large amount of experimental data is already available. In this section the model will be evaluated with experimental results from the literature.

Position dependent sizes of clones with increased Dpp signaling.

When clones with increased Dpp signaling are generated, they grow larger in the lateral regions than in the medial part (Martin-Castellanos and Edgar, 2002). Furthermore, clones with decreased Dpp signaling survive better laterally than medially (Burke and Basler, 1996). A common explanation for these findings is that the medial cells are more competitive than the lateral cells because they receive higher levels of Dpp (Moreno et al., 2002). Therefore, a clone with a fixed level of Dpp signaling is hindered more when growing in the medial part than when growing more laterally. Our model may offer an additional, alternative explanation.

A clone is stretched more and compressed less when growing laterally than when growing medially. Therefore, it grows faster laterally as long as its level of Dpp signaling is fixed. We expect that both competition and differences in compression contribute to the difference of size among different clones.

Non-uniform growth in wings with homogeneous Dpp signalling

Discs with homogeneous Dpp signaling are expanded along the dorsoventral boundary (Martin-Castellanos and Edgar, 2002; Nellen et al., 1996; Rogulja and Irvine, 2005). According to our model, the total growth factor activity in these discs is highest along the dorsoventral boundary, thus accounting for the expansion along this boundary. Furthermore, it has been found that discs with homogeneous Dpp signaling do not show uniform growth. Instead the growth rate of cells in the lateral regions, close to the dorsoventral boundary, is higher than the growth rate of cells in the medial part of the disc (Rogulja and Irvine, 2005). According to the model, the high growth factor activity along the dorsoventral boundary will promote additional growth along the whole boundary. This stretches the regions further away from the dorsoventral boundary. This stretching pulls the cells along the dorsoventral boundary toward the center of the disc. The cells in the center are thus being compressed. The closer the cells are located to the center, the more they are compressed and the more growth is inhibited, thus leading to the observed differences in growth rate.

Non-autonomous stimulation of cell proliferation by clones with modified Dpp signaling.

The Dpp pathway can be activated locally by expressing a constitutively active form of one of its receptors (thickveins (tkv^{Q-D}) (Nellen et al., 1996). Recently, it has been shown that activating the Dpp pathway in clones in this way can stimulate transient non-autonomous cell proliferation. When inhibiting the pathway, similar effects were seen (Rogulja and Irvine, 2005).

We modeled clones with increased Dpp activity as a region with increased Dpp activity compared to its surrounding tissue with lower homogeneous Dpp activity. In that case, the cells with high Dpp signaling initially grow faster than the surrounding cells, thus stretching them. As in the wild-type situation at the start of growth, the stretching is highest in the cells closest to the region with high Dpp signaling and therefore growth is induced in these cells (Fig. 4B,C,S1B). This non-autonomous growth increases the stretching in the cells further away from the clone, which will increase their growth. Therefore, after some time, growth in the cells surrounding the clone will be homogeneous again, comparable with the situation in the wild type disc (Fig. 4C-E). Thus, the model accounts for the non-autonomous effect as well as for the observation that it only occurs transiently.

Clones with decreased Dpp activity were modeled in a similar way. The cells surrounding the clone get stretched between the slow growing cells in the clone and the faster growing cells further away from the clone. Therefore growth is also induced non-autonomously in cells surrounding clones with decreased Dpp signaling (Fig. S1F), which is again in agreement with the data (Rogulja and Irvine, 2005).

Non-autonomous effects on cell proliferation were also assessed for clones in which growth is increased by overexpressing CyclinD and Cyclin-dependent kinase 4 (Cdk4) instead of by increased Dpp signaling (Rogulja and Irvine, 2005). The non-autonomous proliferation was not observed in that case, even though this would in principle be expected based on the model. However, cell divisions are only slightly increased in these clones and apoptosis is increased (Rogulja and Irvine, 2005), which is generally accompanied by basal extrusion (Gibson and Perrimon, 2005; Shen and Dahmann, 2005). Therefore, it seems as if co-expression of CyclinD and Cdk4 causes only very little net overgrowth at the stage measured. For such clones we expect the non-autonomous stimulation of proliferation to be less pronounced and to occur at a relatively late point in time (Fig. S1C and D), which may explain why it has not been observed.

Robustness of size determination against changes in *dpp* transcription

The determination of wing disc size is relatively robust against increases in *dpp* transcription in a pattern approximating its normal pattern (Morimura et al., 1996). In our model, robustness against variations in *dpp* transcription can easily be achieved if it is assumed that the effect of Dpp on growth in the medial part of the disc is saturated. Then, changes in the absolute Dpp levels do not have any effect as long as the Dpp concentration is

sufficient to exert maximum stimulation of growth. If in contrast a continuing linear increase in stimulation of growth is assumed, a doubling of Dpp increases the wing disc size with 13% when otherwise the parameters presented in Fig. 4 are used (Table S1). The final robustness of the system could be achieved by a combination of saturation effects and the robustness displayed by the model itself.

Further experimental results

Experimentally induced alterations in cell proliferation are often compensated for by changes in cell size, such that the final wing disc size is not changed (reviewed in (Potter and Xu, 2001)). This suggests that wing disc size is not a function of cell numbers. In the model, the wing disc is considered as an elastic sheet with certain mechanical properties. As long as the mechanical properties of the tissue as a whole are not influenced by cell size, the final disc size is indeed not a function of cell numbers according to the model.

Lastly, the model predicts that stretching occurs in the peripheral regions. Therefore it also predicts that, upon cutting the disc from the end toward the middle, tissue at both sides of the cut moves apart. In wound healing experiments, this was indeed observed (Fig. 1B in (Mattila et al., 2005)) and these observations were confirmed in our lab (data not shown).

DISCUSSION

We have presented a model for the determination of final size in the wing imaginal disc. With the use of numerical simulations, we showed that the model naturally leads to uniform growth as was shown experimentally and that it leads to the observed final size of the wing disc. Furthermore, we argued that the model can also account for other experimental data in literature.

Experimentally testable predictions

A number of fundamental biological assumptions underlie the model and they form experimentally testable predictions. Firstly, it is assumed that compression inhibits growth and that stretching stimulates growth. This is not an unreasonable assumption, as similar effects have been observed for other tissues. When studying the effect of solid stress on the growth of cancer cells, which is comparable to compression in the model, it was found that it inhibits growth of all cancer types tested (Helmlinger et al., 1997). Furthermore, for pulmonary artery endothelial cells and for kidney epithelial cells it was observed that tractional stress induces growth (Nelson et al., 2005). Moreover, in our model stretching forces are equivalent to changes in cell shape and changes in cell shape have been shown to affect growth (reviewed in (Huang and Ingber, 2000)). Significantly, for aortic endothelial cells, hepatocytes, and capillary endothelial cells it has been observed that spread cells proliferate more than more rounded cells (Chen et al., 1997; Folkman and Moscona, 1978; Singhvi et al., 1994). On the molecular level, a central player in mechanotransduction appears to be integrin (Alenghat and Ingber, 2002) and it has been

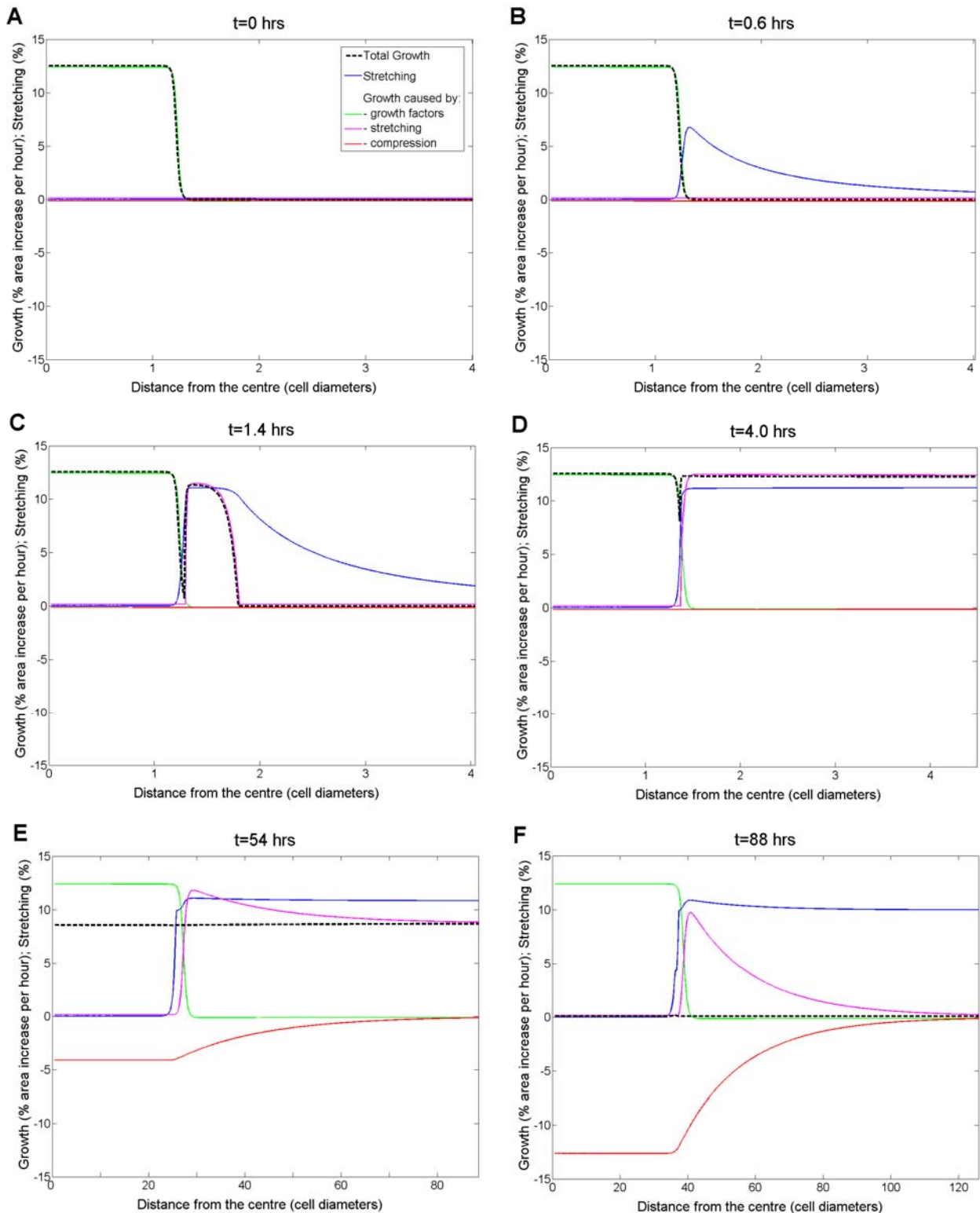


Fig. 4. Different time points of a simulation experiment. Curves were shifted slightly to prevent overlap. The individual steps are discussed in the text. The images are after 0.0, 0.6, 1.4, 4.0, 54.0, and 88.0 hours have been simulated. Total growth (black dotted) is shown as well as the different components of growth which are attributable to different factors (growth factor: green; stretching: magenta; compression: red). Furthermore, the extent of stretching is indicated (blue), which is basically the difference between the width and length of a cell. The materials and methods section contains the exact calculations to obtain the stretching. The length in cell diameters is used as an absolute length scale and denotes an average cell diameter. It is therefore for example possible that the number of cells increases even though the wing disc size in cell diameters, as given in the figure, does not change. Note that the growth factor distribution does not change as the wing disc size increases. This is because its distribution is assumed to be scaled, as described in the text.

proposed that integrin, linked to the extracellular matrix, is involved in mediating tension induced cell proliferation (Schwartz and Ginsberg, 2002). For wing disc cells the effects of stretching and compression remain to be studied.

Secondly, it is assumed that there is a growth factor gradient present in the dorsoventral axis and that both Dpp and this second growth factor are required to induce growth. A candidate protein for this growth factor is Wingless (Wg). *wg* is expressed in the dorsoventral boundary and forms a gradient (Baker, 1988; Couso et al., 1994; Strigini and Cohen, 2000). Reduced Wg activity leads to a reduction in the size of the wing (Couso et al., 1994; Neumann and Cohen, 1996) and an increased production of Wg in clones leads to additional growth (Diaz-Benjumea and Cohen, 1995; Ng et al., 1996). Furthermore, ectopic overactivation of *wg* stimulates a net increase in cell proliferation in the proximal part of the wing (Giraldez and Cohen, 2003). This would be expected by the model for overactivation of the second growth factor, in analogy with non-uniform growth in a mutant with uniform Dpp activity. However, under certain circumstances Wg also seems to function as a growth inhibitor, complicating the situation (Johnston and Edgar, 1998; Johnston and Sanders, 2003). Furthermore, other signals, such as those mediated by Notch, seem to be involved in inducing growth as well (de Celis and Garcia-Bellido, 1994; Diaz-Benjumea and Cohen, 1995; Giraldez and Cohen, 2003).

Thirdly, it was assumed that the growth factor activity gradient is scaled. We also performed simulations with a non-scaled gradient. While these simulations still showed termination of growth, the deviations from uniform growth were generally larger than with a scaled gradient. When visualizing the Dpp activity gradient by assessing the phosphorylation of Mothers against Dpp (Mad), it was observed that the gradient adjusted to compartment size when this was experimentally altered (Teleman and Cohen, 2000). It remains to be tested whether this scaling is also present at different time points of normal wing disc development and whether the activity of the second growth factor, if indeed present, is scaled as well.

Fourthly, it was assumed that stretching only induces growth above a certain threshold. In fact, for the model to work it is not necessary that this is true for all cells. It is also sufficient if stretching induces growth above a certain threshold in the hinge regions (most peripheral regions) of the disc. Alternatively, it would also be sufficient if the peripodial membrane is stretched to a certain degree during growth. This membrane covers the columnar layer of the disc, where the Dpp gradient is formed (Gibson et al., 2002). Since the columnar layer and the peripodial membrane are connected, stretching in the peripodial membrane could compress the columnar layer to some degree. This compression could lead to a stress distribution in the disc, which can contribute to terminating growth in the absence of a stretching threshold by affecting growth in the columnar layer itself (Fig. S2). Thus, while it is

essential that some stretching remains after growth, the model allows for different locations of the cells in which stretching only induces growth above a certain threshold. Even though the presence of stretching is suggested by the moving apart of both sides of a cut through the wing disc (Mattila et al., 2005), more precise measurements on its extent and distribution are required. Such experiments could also help to refine the model, since the predicted distribution of stretching depends on its precise formulation (Fig. S3).

Comparison with a related model

The model presented here shows some similarities with a model proposed by Shraiman (Shraiman, 2005). He showed that a clone, which grows at a different rate than the surrounding tissue, is subject to mechanical stress. Supposing a dependence of the rate of cell division on local stress he then obtains an "integral-feedback" mechanism, which stabilizes uniform growth (Shraiman, 2005). This is similar to the way uniform growth is achieved in our model. On the other hand, our model also shows fundamental differences to that of Shraiman. First, it does not only consider the effect of forces, but also considers the effect of growth factors. Second, it explicitly takes into account the geometry of the wing disc, including boundary conditions and sources of growth factors. As a result, our model cannot only account for homogeneous growth, but also for the termination of growth, in contrast to the model proposed by Shraiman. Interestingly, Shraiman mentions that compression of cells within a layer can be at least partially relieved by the buckling of the cell layer out of the plane (Shraiman, 2005). In this light it would be an interesting possibility that the building up of mechanical stress during normal growth, such as predicted by our model, may contribute to the folding of wild type wing discs.

Implications

Dpp does not only function as a growth factor, it also functions as a morphogen to mediate patterning of the wing disc (Lecuit et al., 1996; Nellen et al., 1996). It thus seems to connect patterning and growth, which is generally important to ensure the proper development of a multicellular organism. It has been a paradox though that patterning depends on differences in Dpp activity among different regions of the disc, whereas growth occurs uniformly throughout the disc, even though it is induced by Dpp. In the model presented here, a scaled Dpp activity gradient is needed to induce uniform growth. It is therefore very efficient that Dpp is used to regulate both patterning and growth and it guarantees a tight coupling of both processes. This raises the possibility that such coupling of patterning and growth might more generally occur during development.

The model is formulated for the wing imaginal disc. In principle, very similar models could be applicable for other round tissues in which growth factor concentrations are highest in the center. For example, in the leg imaginal disc of *Drosophila* both Dpp and Wg seem to be necessary to

induce growth, and, even though they do not show the same expression pattern as in the wing disc, their combined activity is highest in the center of the disc (Campbell et al., 1993; Serrano and O'Farrell, 1997). More generally, there may be other systems in which regulation of final form is achieved as a result of a growth factor distribution in combination with mechanical forces, which are automatically generated in the specific geometry upon stimulation of growth by growth factors.

MATERIALS AND METHODS

Assumptions

Beside the biological assumptions discussed in the results section, the following assumptions were made in order to facilitate the modeling:

- It is assumed that the disc is radially symmetrical, which implies that it does not include the notum (see Fig. 2). Including the notum would require an extension of the model, for example by assuming that growth in the blade stretches the notum, which then causes the notum to grow. Furthermore, the other part of the wing disc, the wing blade and hinge region (Fig. 1), is not completely round either. A more oval shape could for example be obtained by assuming that Dpp has a longer signaling range than the second growth factor. Finally, the net growth factor concentration (combination of Dpp and a second growth factor) is distributed in a circular manner. A model with a more square-like distribution of growth factors, which could be the result of combining two perpendicular gradients, would probably also yield a round or almost round disc.
- The disc is considered to be an elastic sheet; individual cells are not explicitly considered. This assumption is made since the cytoskeletons of adjacent epithelial cells are connected to each other via cell adhesion molecules like cadherins, allowing for the development of mechanical stress within the epithelium (Braga, 2000; Chen et al., 2004).
- The density of cell material remains constant over the disc. This implies that compression cannot cause cells to become denser. We expect however that including changes of density would not change the simulation results fundamentally, but that it would merely require more growth in the center of the disc (increase in mass) before the same stretching occurs in the more peripheral regions.
- Growth is omni-directional in the model. This is in agreement with observations that mitotic orientations are at random within clusters of dividing cells (Milan et al., 1996) and that cells do not seem to be relocated after division (Baena-Lopez et al., 2005). However, preferential orientations of cell divisions have been observed as well (Baena-Lopez et al., 2005). If random orientations are not assumed, larger deviations from uniform growth may arise, dependent on the nature of its regulation.
- Apoptosis is not included in the model, since apoptosis in wild-type discs is a rather rare phenomenon during larval development (Milan et al., 1997).

- The wing disc is modeled as a flat disc, even though the wing disc becomes folded towards the end of the third instar stage. We expect that extra growth (increase in area) does not only lead to stretching, but also to extra folding, in such a way that stretching and bending forces are in equilibrium. Since the bending may lead to a reduction in compression of the center compared to a situation where the disc would be flat, growth might cease at a later time point, without changing the principle behind the termination of growth.
- Initially, no forces are present in the disc.
- It is assumed that the growth factor concentration is high in the center of the disc and then drops to zero in the peripheral regions. For the model, it is not necessary that growth factors really are absent in the peripheral regions, as long as there is not enough growth factor present to induce growth in the absence of stretching. Otherwise, growth would not stop. Furthermore, the model also yields uniform growth and termination of growth when a shallower growth factor gradient is used than the one for the generation of Fig. 4.

General simulation approach

The wing imaginal disc is subdivided into small concentric rings (Fig. 2). For each ring, growth (increase in area) is calculated for a small time step δt . Then, new dimensions are calculated by redistributing the new area. Based on these new dimensions, a new growth factor distribution is calculated. Furthermore, new stretching and compression forces are calculated, which are then used in order to calculate growth for the next δt , which is iterated further until a steady state is reached.

Calculation of growth

The results in Fig. 4 were obtained by using the following equations to calculate growth in each of the rings (relative increase in area):

$$\Delta G_{gf} = c_1 * \tanh(c_2 * Gf) * \Delta t$$

$$\Delta G_{str} = (|Grn| - c_3) * c_4 * \Delta t (\Delta G_{str} \geq 0)$$

$$\Delta G_{comp} = -(c_5 * \exp(Comp(n) * c_6) - c_5) * \Delta t$$

$$\Delta G_{tot} = \Delta G_{gf} + \Delta G_{str} + \Delta G_{comp} (\Delta G_{tot} \geq 0)$$

Here G_{gf} , G_{str} , G_{comp} , and G_{tot} denote growth caused by the activity of growth factors, growth caused by stretching, growth (inhibition) caused by compression and total growth respectively. G_{tot} and G_{str} cannot be negative. Gf denotes the growth factor distribution, Grn the growth needed to abolish stretching, and $Comp$ the compression exerted by stretching of the more peripheral rings. Initially, Grn and $Comp$ are 0 everywhere. Afterward, they are calculated as described below. The growth factor distribution is always calculated as described below. c_1 - c_6 are constants, with values 0.13 hour^{-1} , 2.0, 0.1, 10 hour^{-1} , 0.0013 hour^{-1} , and $0.5 \text{ cell diameter}^{-1}$ respectively.

The inhibition of growth by compression was chosen to occur in an exponential manner, since this provides more robustness to the model against variation in Dpp activity than a linear relationship. However, the model would also be in agreement with the experimental data when other relationships are chosen.

The parameter values are not known experimentally and were selected in such a way that final disc size is about 50,000 cells and that the developmental time does not exceed 88 hours. However, different parameter combinations fulfill these requirements. Furthermore, specific parameter combinations yield other results when the mathematical relationships among variables are changed. Therefore, the model cannot be used to predict values of parameters.

Calculation of new dimensions

The dimensions are calculated by redistributing the surface, starting from the center ring ($n=1$). Naturally, the r_1 for one ring is equal to the r_2 of the adjacent ring closer to the center ($r_1(n)=r_2(n-1)$; Fig. 2). For the r_2 of ring n the following equation is used:

$$r_2(n) = ((r_{2old}(n)^2 - r_{1old}(n)^2) * (1 + \Delta G_{tot}(n)) + r_1(n)^2)^{1/2},$$

where r_{2old} and r_{1old} are the r_2 and r_1 of a certain ring before the latest growth step.

Calculation of new growth factor distribution

The growth factor distribution is calculated as follows:

$$Gf(n) = -0.5 * \tanh(c_7/r_2(N) * r_2(n) - c_7 * c_8) + 0.5,$$

where $r_2(N)$ is the r_2 of the outer ring and therefore the radius of the disc and c_7 and c_8 are constants with values 100 and 0.3 respectively.

Calculation of stretching and compression

Stretching (Str) is calculated as follows.

$$sur_{t0}(n) = \pi * (r_{2t0}(n)^2 - r_{1t0}(n)^2)$$

$$l_{t0}(n) = r_{2t0}(n) - r_{1t0}(n)$$

$$sur(n) = \pi * (r_2(n)^2 - r_1(n)^2)$$

$$l(n) = r_2(n) - r_1(n)$$

$$Str(n) = (sur(n)/l(n)^2) / (sur_{t0}(n)/l_{t0}(n)^2) - 1,$$

where sur_{t0} , sur , l_{t0} , and l stand for the surface of a certain ring at $t=0$, the present surface of the ring, the width of the ring at $t=0$, and the present width of the ring. If cells have the same width and length at $t=0$ (no forces present), stretching basically means the different between the width and the length of a cell.

Once the stretching is calculated, the compression can be calculated as well:

$$Comp(n) = \sum_{m=n+1}^{m=N} Str(m) * (r_2(m) - r_1(m)).$$

Furthermore, the growth that would be needed to abolish the stretching in a certain ring (Grn) is calculated as well:

$$r_{2n}(n) = r_{2t0}(n) / r_{1t0}(n) * r_1(n)$$

$$Grn(n) = (r_{2n}(n)^2 - r_1(n)^2) / (r_2(n)^2 - r_1(n)^2) - 1,$$

where r_{2n} denotes the r_2 needed to abolish stretching, given the r_1 of the ring.

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Parameter	Disc radius after doubling the parameter value		Disc radius after halving the parameter value	
	Absolute length (cell diameters)	Relative length (-)	Absolute length (cell diameters)	Relative length (-)
c ₁	143	1.13	108	0.86
c ₂	128	1.01	116	0.92
c ₃	64	0.51	247	1.96
c ₄	128	1.01	124	0.98
c ₅	107	0.85	146	1.15
c ₆	63	0.50	253	2.0
c ₇	127	1.01	125	0.99
c ₈	218	1.73	104	0.83

Table S1. Robustness of the model against doubling and halving parameter values. The relative length is calculated by dividing the obtained absolute length by the absolute length under the reference conditions (126 cell diameters). Even though the model is fairly robust against changing a number of its parameters, we could imagine that additional robustness is achieved in the biological system by mechanisms, which are not included in the model. For example, the growth factor gradient may be established in a robust way.

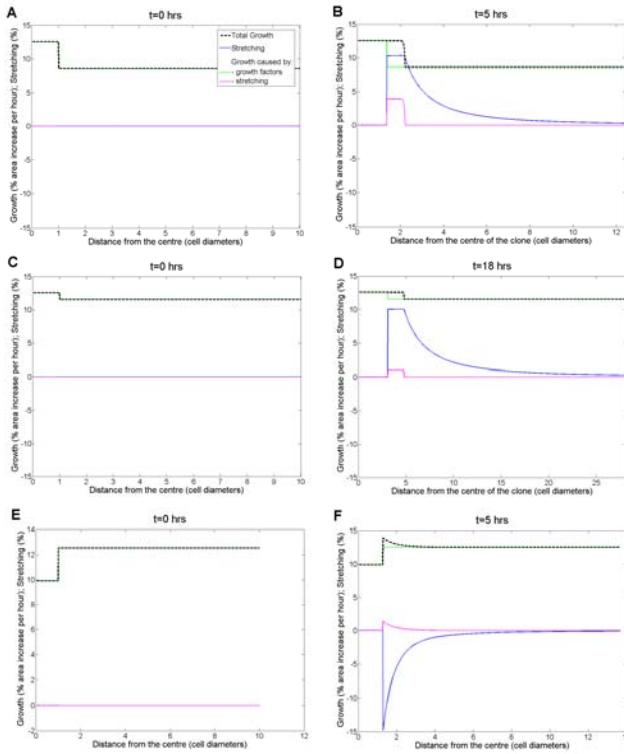


Fig. S1. Simulating clones with increased or decreased Dpp signaling. A and B: Two snapshots of a simulation experiment where the center 10% of the tissue has maximum Dpp activity and the surrounding tissue 0.4 * maximum activity. C and D: The same simulation experiment as for A and B, but the surrounding tissue contains 0.7 * maximum Dpp activity. E and F: The center 10% of the tissue has 0.5 * maximum activity, whereas the surrounding tissue contains maximum activity. For A, B, C and D, the equations, other than the Dpp activity distribution, and parameter values used are the same as for the reference model, except that $c_5 = 0 \text{ hour}^{-1}$ (no compression) for simplification. For E and F two additional parameter values were changed: $c_3=0.01$ and $c_4=0.1 \text{ hour}^{-1}$.

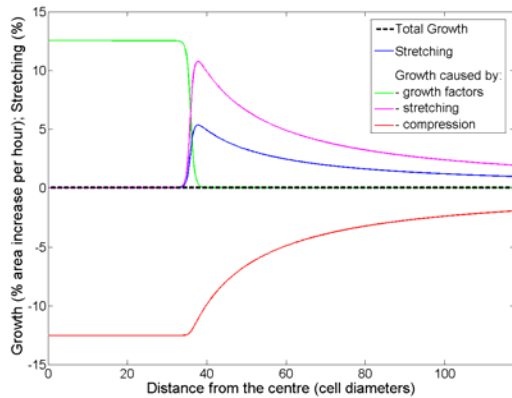


Fig. S2. Situation at the end of growth in the presence of constant stretching in the hinge

region or in the peripodial membrane. Stretching in the hinge region or the peripodial membrane was modeled by assuming that the outer ring is surrounded with a ring which is stretched, such that the total compression felt by each ring is increased with a constant. Furthermore, the threshold above which stretching can stimulate growth was assumed to be 0.

The compression is thus calculated as follows:

$$\text{Comp}(n) = \sum_{m=n+1}^{m=N} (\text{Str}(m) * (r_2(m) - r_1(m))) + c_p,$$

with $c_p = 2$ cell diameters. The other parameter values used are the same as for the reference model, except that $c_3=0$, $c_4 = 2 \text{ hour}^{-1}$, $c_5=0.003 \text{ hour}^{-1}$, and $c_6=1 \text{ cell diameter}^{-1}$.

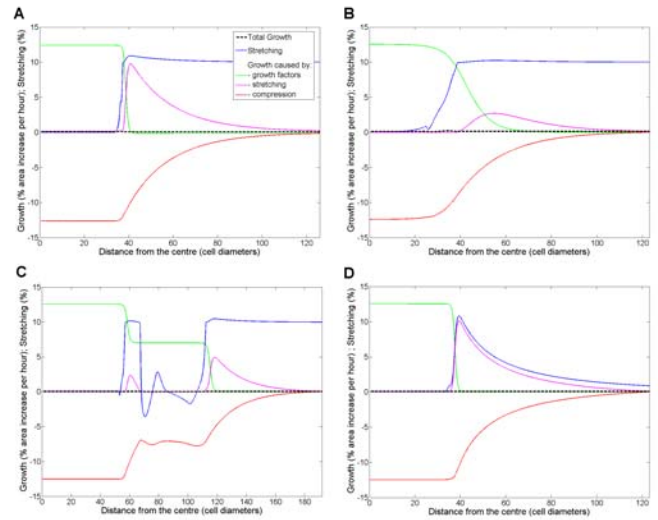


Fig. S3. Distribution of mechanical stress after completion of growth for different modulations of the model. A: Reference model. B: Model with a more shallow Dpp distribution (c_7 has a value of 10 instead of 100). C: Model with two growth factor activity thresholds (comparable with a threshold for *spalt* and one for *optomotor blind transcription (omb)* (Nellen et al., 1996)). The following equation was used to calculate the Dpp distribution: $\text{Gf}(n) = -0.35 * \tanh(c_7/r_2(N) * r_2(n) - c_7 * c_8) - 0.15 * \tanh(c_7/r_2(N) * r_2(n) - c_7 * c_9) + 0.5$, where c_7 , c_8 and c_9 are constants with values 100, 0.3 and 0.6 respectively. D: Model in which the ratio between the values of a number of constants have been changed (c_3 - c_5 have values of 0.08, 0.04 hour^{-1} , and 1 hour^{-1} instead of 0.1, 10 hour^{-1} , and 0.0013 hour^{-1} respectively).