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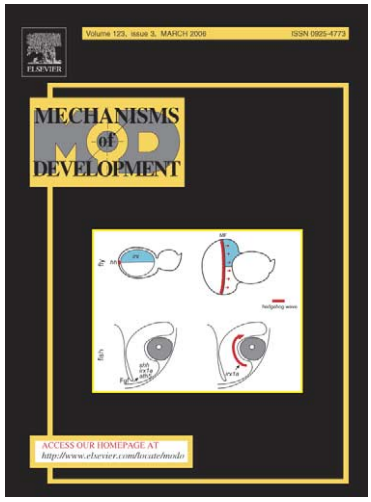

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Model for the regulation of size in the wing imaginal disc of *Drosophila*

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ABSTRACT

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.

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. Because of its relatively simple structure and its accessibility for genetic manipulations, the wing imaginal disc of *Drosophila* has most widely been used as a model system to study the regulation of growth. Genes that appear to be crucial for its regulation, have also been found to be expressed during development in other tissues in other organisms, raising the possibility that common mechanisms are employed in different species. Despite extensive experimental investigation of the imaginal discs, mechanisms underlying the determination of size remain elusive. Therefore, there is a need for models, which can explain the available data and possibly even inspire entirely novel experimental approaches.

Drosophila imaginal discs are 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., 1996b). The adult wing is produced by the eversion of the wing disc. Upon eversion, intervein regions divide once. Vein and intervein cells undergo two mitotic rounds, but only one full cell cycle (Milan et al., 1996a). Therefore, the size of the adult wing is predetermined by the final size of the wing disc (Day and Lawrence, 2000). Wing disc size seems to be mostly regulated disc autonomously, since transplantation of early discs into the abdomen of adult flies results in discs with normal size (Bryant and Levinson, 1985; Jursnich et al., 1990).

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 roughly uniformly throughout the disc (Milan et al., 1996b).

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.

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 AND DISCUSSION

The model

There are a number of biological assumptions underlying the model. Firstly and most fundamentally, it is assumed that net 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. Note that the term net growth here denotes increase in epithelial surface area, and does not distinguish between changes in average cell size and changes in cell number. 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.

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. 2A and B). Growth in the central part naturally results in stretching of the peripheral regions (Fig. 2B). 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. In this way,

final disc size is determined by a combination of the effect of growth factors, by the threshold above which growth is induced by stretching, and by the effect of compression on growth.

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 Supplemental Text. Here, the simulation results will be discussed. Fig. 3 shows different time points of a simulation experiment. A movie of the same simulation experiment can be found in the Supplemental Data (Movie S1).

At the start of the simulation, growth only occurs in the center of the disc (Fig. 3A). This growth stretches the directly adjacent regions (Fig. 3B), causing them to grow (Fig. 3C). The stretching then spreads to the most peripheral parts, such that growth is induced in these regions as well (Fig. 3C and D). This results in homogeneous growth throughout the disc at a time point when the disc has hardly increased in size yet (Fig. 3D). During the whole growth process, the peripheral region stays slightly stretched, thus compressing the center (Fig. 3D and E). In late discs, this stretching is more pronounced in the regions adjacent to the center than in the most peripheral regions

(Fig. 3E and 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. 3E). Eventually, growth ceases completely when the disc has a radius of 126 cells, corresponding to about 50,000 cells (Fig. 3F). Consistent with cell number counts in wing discs (Bryant and Levinson, 1985), growth rate is high (cell-number doubling time of about 6 hrs) during the first 40-50 hrs of disc growth and drops to almost zero during the subsequent 30-40 hrs.

The simulation experiment shown in Fig. 3 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.

In order to get an impression of the robustness of the model to changing its parameter values, each parameter value was halved and doubled respectively. As can be seen in Table 1, the model is fairly robust against changing most of its parameters, but it is not very robust with respect to three of them. The first one concerns the position of the steepest part of the growth factor gradient (c_8), the second one concerns the stretching threshold above which stretching causes growth (c3), and the third one is a measure for the reaction of cells to compression (c_6). Given the low variability in wing disc sizes, especially of those within a single larva, we expect that additional precision is achieved by mechanisms, which are not included in the model. For example, the growth factor gradient may have a low variability among different wing discs. On the other hand, a limited robustness against a parameter also offers a possibility for the realization of differences in size among different organs, if the size is determined in a similar way. For example, haltere discs are similar to wing discs, but they are much smaller. This seems to be a least partially achieved by a restricted Dpp distribution, which is caused by an increased amount of the Dpp receptor Thickveins (Tkv). This increased amount of Tkv in turn is mediated by the Hox gene *ultrabithorax (ubx)*, which is expressed in the haltere disc, but not in the wing disc (Crickmore and Mann, 2006). Thus, differences in size among different kinds of imaginal discs seem to be mediated in part by affecting the Dpp distribution and this indeed affects one of the parameters in the model (c_8) to which final disc size is relatively sensitive.

Lastly, it should be noted that the model is robust against changes in maximum Dpp activity. However, this is not a feature that is inherent to the model, but it depends on the exact equations and parameters chosen. An increased robustness against changes in maximum Dpp activity is generally accompanied by a decreased robustness against parameters concerning the reaction of cells to forces and vice versa. We chose for a relatively high robustness against changes in maximum Dpp activity, since it has been shown that the determination of wing disc size is robust against increases in *dpp* transcription in a pattern approximating its normal pattern (Morimura et al., 1996).

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 (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 (see Supplemental Data). 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. 3B, C and S2B). 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. 3C-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 (see Supplemental Data). 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. S2F), 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 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. S2C and D), which may explain why it has not been observed.

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.

Furthermore, according to the model, it would be expected that a reduction of growth in the center of the disc automatically leads to a reduction of growth in the peripheral regions. Indeed, when the size of the wing blade was decreased by down-regulating *vestigial* (*vg*) expression, nonautonomous reductions in surrounding WT territories were observed along all axes of growth (Baena-Lopez and Garcia-Bellido, 2006). 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). On the other hand, the model predicts that the central region of the disc becomes compressed. The increased thickness of the (columnar layer of the) wing disc could be seen as an indication that compression indeed occurs.

Discussion

We have presented a model for the determination of final size in the wing imaginal disc. In the model, growth is negatively regulated by mechanical stresses, which are automatically generated as a result of growth rate differences in an elastic tissue. 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 assumptions

A number of fundamental biological assumptions underlie the model and they form experimentally testable predications. 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 proposed that integrin, linked to the extracellular matrix, is involved in mediating tension induced cell proliferation (Schwartz and Ginsberg, 2002). Alternatively, the molecular response to stretching and compression may also be mediated indirectly by differences in contact inhibition. In the center of the disc, the cells become columnar, whereas cells in the most peripheral regions stay cuboidal (McClure and Schubiger, 2005), which could partially be caused by the building up of mechanical stress, such as predicted by our model. In comparison with a cuboical cell, a larger part of the cell surface of a collumnar cell is in contact with neighbouring cells, which could lead to increased contact inhibition. A candidate for mediating contact inhibition could be Fat, which is an atypical cadherin that strongly inhibits growth (Buratovich and Bryant, 1997; Garoia et al., 2005), at least partially through the hippo tumor-suppresor pathway (Willecke et al., 2006).

For wing disc cells the effects of stretching and compression, including underlying mechanisms, remain to be studied. The most direct way of assessing the effects of stretching and compression would be by direct mechanical manipulation of the wing discs. This approach would however require new methods to overcome the relative inaccessibility of growing imaginal discs. An alternative would include using cell cultures, but it is of course not clear whether culture cells respond in the same way to mechanical forces as cells which are integrated in a tissue.

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 periopodial 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 (see Supplemental Data). 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 (see Supplemental Data).

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.

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FIGURE LEGENDS

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.

Fig. 2. 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.

Fig. 3. 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.

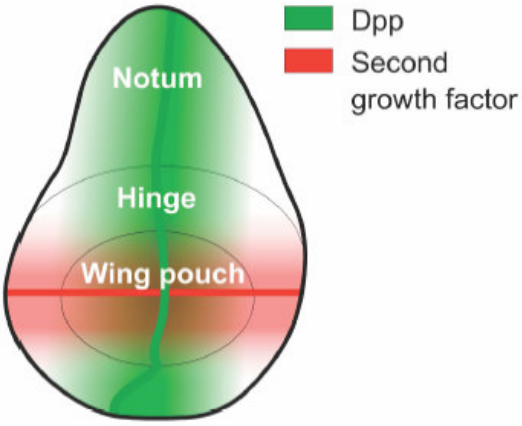
TABLE LEGEND

Table 1. 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). In the supplemental text the exact meaning of the different parameters can be found.

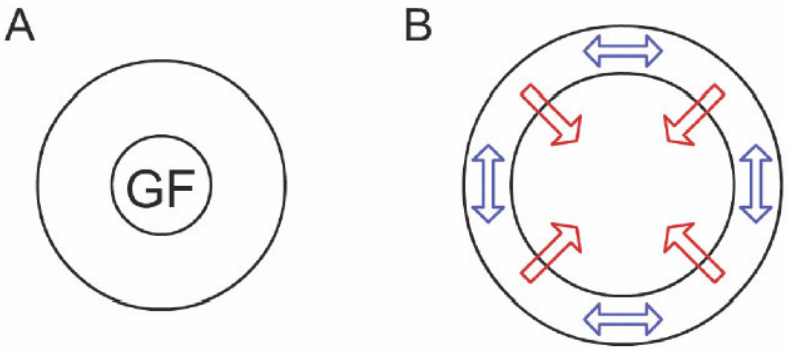
Table 1

Parameter with an indication of its physical meaning		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	Maximum growth factor activity	143	1.13	108	0.86
c_2	Sensitivity to growth factor conc. changes	128	1.01	116	0.92
c_3	Stretching threshold	64	0.51	247	1.96
c_4	Effect of stretching on growth	128	1.01	124	0.98
c_5	Effect of compression on growth	107	0.85	146	1.15
c_6	Effect of compression on growth	63	0.50	253	2.0
c_7	Steepness of growth factor gradient	127	1.01	125	0.99
c_8	Position of steepest	218	1.73	104	0.83

	part of the growth factor gradient				

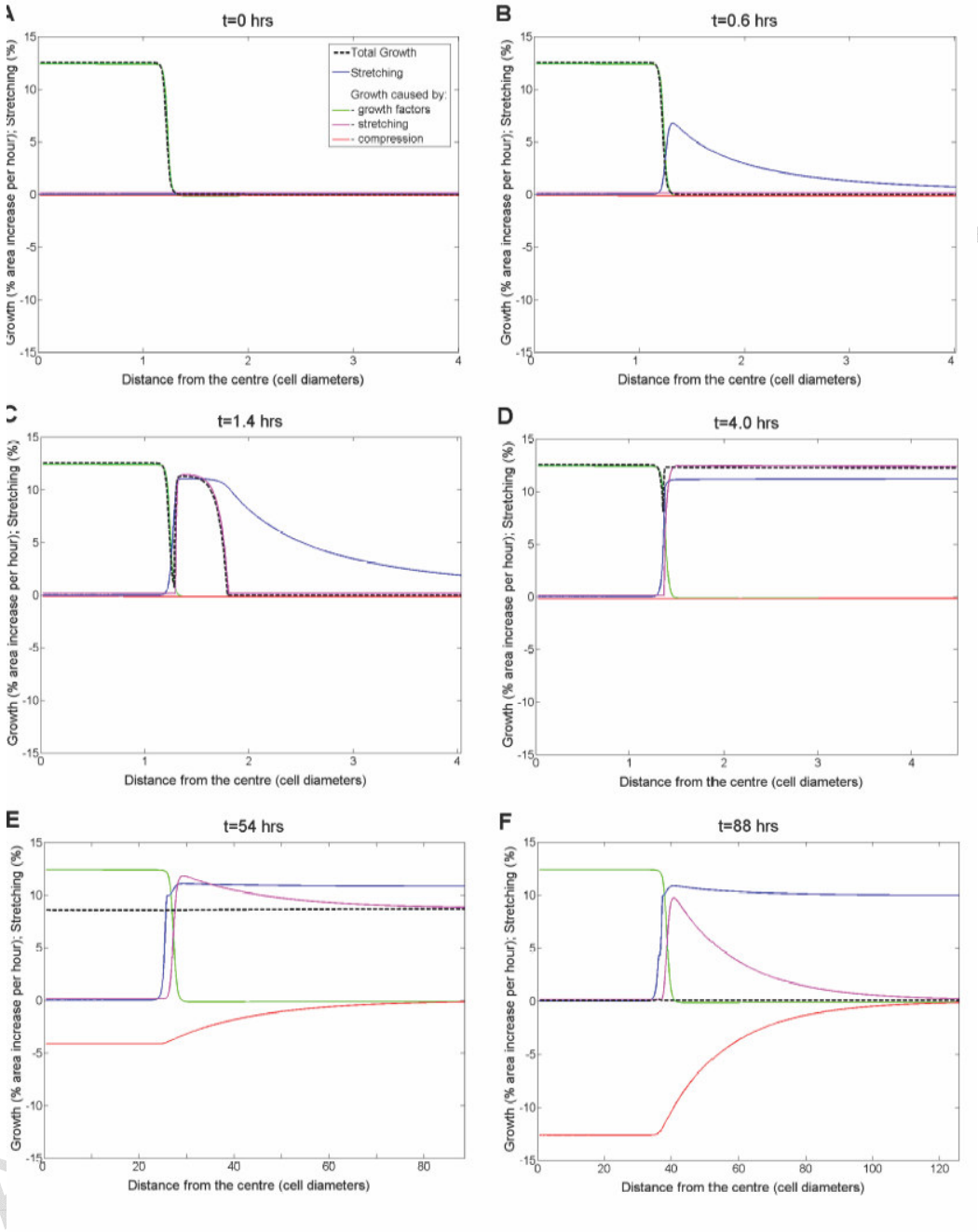


Aegerter-Wilmsen et al., Fig. 1



Aegerter-Wilmsen et al., Fig. 2

Figure 3


A
t=0 hrs
B
t=0.6 hrs
C
t=1.4 hrs
D
t=4.0 hrs
E
t=54 hrs
F
t=88 hrs
Total Growth
Stretching
Growth caused by:
growth factors
stretching
compression
Growth (% area increase per hour); Stretching (%)
Distance from the centre (cell diameters)