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Sizing it up: The mechanical feedback hypothesis of organ growth regulation

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Abstract

The question of how the physical dimensions of animal organs are specified has long fascinated both experimentalists and computational scientists working in the field of developmental biology. Research over the last few decades has identified many of the genes and signaling pathways involved in organizing the emergent multi-scale features of growth and homeostasis. However, an integrated model of organ growth regulation is still unrealized due to the numerous feedback control loops found within and between intercellular signaling pathways as well as a lack of understanding of the exact role of mechanotransduction. Here, we review several computational and experimental studies that have investigated the mechanical feedback hypothesis of organ growth control, which postulates that mechanical forces are important for regulating the termination of growth and hence the final physical dimensions of organs. In particular, we highlight selected computational studies that have focused on the regulation of growth of the *Drosophila* wing imaginal disc. In many ways, these computational and theoretical approaches continue to guide experimental inquiry. We demonstrate using several examples how future progress in dissecting the crosstalk between the genetic and biophysical mechanisms controlling organ growth might depend on the close coupling between computational and experimental approaches, as well as comparison of growth control mechanisms in other systems.

Keywords

Computational and mathematical modeling; intercellular signaling; limb development; mechanobiology; intercellular signaling; *Drosophila* wing disc

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1. Introduction

1.1 The questions of organ size control

Size control at the level of both organs and organisms has long fascinated biologists due to the large variation of sizes in the animal kingdom and the medical importance of growth control in many diseases such as cancer and genetic birth defects. Early work exemplified by D'Arcy Thompson's treatise "On growth and form" focused on biophysical principles of morphogenesis [1]. The last few decades have witnessed significant advances in identifying biochemical signaling pathways involved in growth control regulation, but an integrated, holistic view of how information on the physical dimensions of tissues is transduced by biochemical signaling pathways to regulate cell growth and homeostasis is still lacking [2]. The question of size control has been approached from multiple angles: physiology, genetics, developmental biology, biophysics, and mathematical and computational modeling [3–13]. Computational studies play a role not only in better understanding mechanisms of development but also in integrating information between different biochemical and biophysical phenomena into an unified, predictive model [14,15].

Computational modeling has played a significant role in experimental inquiry through the development, refinement and testing of the mechanical feedback hypothesis, which postulates that mechanical forces play an important role in coordinating growth between cells within tissues and as well as modulating instructive inputs from growth factors and morphogens. This hypothesis views mechanical forces not merely as physical constraints, but also as information-providing regulatory inputs into the calculations performed by cells during development. Despite the appreciation of mechanical stress as an integral factor controlling tissue size and an expanding understanding of the gene regulatory networks that control growth [16–21], decisive experimental tests are still needed to elucidate how the signaling mechanisms integrate mechanical constraints with biochemical signals in specific organs. Here, we focus on a select set of computational and experimental studies that have helped shape the mechanical feedback hypothesis of organ growth. Our discussion centers on the particular context of *Drosophila* wing disc development, which has served as a paradigm for growth control research.

1.2 Wing discs as a model organ for growth control

Our understanding of size control at the level of individual organs or the whole body is most highly advanced in the "golden insect" *Drosophila melanogaster* [22]. While the developmental specifics for a particular organ are unique, there is an overarching conservation of signaling pathways and regulatory mechanisms that are informative toward human development and disease ontogenesis [23–26].

The adult wings of *Drosophila* are derived from imaginal discs that are specified during embryogenesis and proliferate throughout larval development (also called the imago stage) to expand from approximately 50 to 50,000 cells, a thousand-fold increase, over the course of five days (Fig. 1A, B). This developmental period covers three sequential instars or moltings that occur during larval development [27–29]. The wing imaginal disc consists of an epithelial monolayer sac with a lumen. As development proceeds, multiple folds form

within the monolayer (Fig. 1B–B”). The wing blade is derived from the central oval shaped “pouch” of the wing disc, with the cells in the center of the pouch forming a pseudostratified epithelium of highly packed cells. Above the pouch is a squamous epithelium called the peripodial membrane. Historically, the majority of studies in wing disc growth have focused on the size and shape of the pouch region of the wing disc due to the accessibility of imaging a relatively flat portion of the tissue. The pouch also contains the morphogenetic center of the wing disc.

Organ size regulation depends on both intrinsic and extrinsic factors [9]. Intrinsic growth control is the inherent ability of organs within the body to regulate final size based on its genetic program, which each individual cell within the organ contains. In general, it is understood that morphogen signaling pathways are “master architects” coordinating patterning and growth in developing organs [30]. Extrinsic growth control is the influence of systemic signals – hormones and nutrients – on organ development. For example, Insulin Receptor (InR) signaling and the target of rapamycin (TOR) pathways are essential regulators of growth rate and duration. These pathways communicate the nutrient status of the animal and couple nutrition to growth [7,31–33]. Additionally, extrinsic mechanical forces from neighboring tissues can also potentially provide input into the growth potential of the organ. Outstanding questions in the growth control field include the mechanism of size regulation by each modality (intrinsic and extrinsic). Interorgan communication can play an important role in the size control of wing discs [5,34–36]. However, potential cross-talk between intrinsic and extrinsic growth control modalities has not been approached to any significant degree using computational approaches to date [9].

2. Overview of chemical factors regulating growth

Several intercellular signaling pathways impact growth in the *Drosophila* wing disc, including Decapentaplegic (DPP, a TGFβ family member), Wingless (WG)/WNT, Notch, EGFR and Fat-Dachsous (which provides input into the Hippo pathway) [10,37–46]. In particular, DPP and WG belong to a class of molecules called morphogens that are locally secreted and transported across the tissue to regulate growth and the spatial pattern of transcriptional activity and cellular differentiation. These two morphogens define a coordinate axis for the wing with DPP patterning the anterior-posterior (AP) and WG patterning the dorsal-ventral axis (DV) and jointly provide input into the Dachsous/Fat/Hippo signaling pathway (Fig. 1B) [43,47]. Studies in the wing disc have played an important role in establishing the role of morphogen protein gradients in regulating pattern formation and organ size [30,48], which is covered in greater detail by several recent reviews [10,30,37,49]. How cells convert morphogen concentration gradients into the observed spatially uniform pattern of proliferation remains unclear and several competing models have been proposed.

Secreted morphogens have been implicated genetically in growth control, including Wingless (WG), Decapentaplegic (DPP) and Hedgehog (HH). For example, the morphogen DPP is crucial in the size regulation of a developing wing imaginal disc of *Drosophila* along the AP axis. Experiments have shown that insufficient DPP hinders growth, while over expression increases the size significantly [50–54]. The distribution of DPP is

inhomogeneous throughout the wing disc, yet cell proliferation is uniform throughout the organ during later stages of growth [55,56]. An important criteria for a successful model of growth regulation must explain how non-uniform signaling by an inductive signaling gradient results in the observed uniform growth across the tissue [57]. Whether, and how, morphogen gradients are required in growth regulation, however, has become less clear with the finding that intercellular transport of Wingless is not absolutely required [58]. Other intercellular pathways such as Notch (N), Epidermal Growth Factor (EGFR), and Fat-Dachsous/Hippo signaling are also implicated in the regulation of organ growth [42,45,47,59–63]. The complexity of the signaling network therefore demands continually refined computational approaches to capture emergent properties of the regulatory system. Notably, however, recent quantification of growth during early stages of development appears less uniform with higher levels in the morphogenetic center of the pouch, consistent with the idea that compression must build up in the disc over time before beginning to significantly inhibit growth (discussed in greater detail in section 3) [64].

Proposed mechanisms that try to explain this apparent paradox of non-uniform signaling directing relatively uniform growth have been contradicted by the available evidence or still need additional verification experimentally ([37,57,65–67], were reviewed recently in [30]). An early and particularly influential hypothesis of the impact of morphogen activity on tissue growth was proposed by Day and Lawrence [57], which stated that the slope of morphogen gradients gives cells positional information on the size of the organ across that dimension. In this model, the morphogen gradient scales with growth leading to a flattening of the gradient. Growth terminates once the slope drops below a particular threshold value. This conceptual model, supported by logical reasoning and data available at the time, has been influential in guiding research and in forming hypotheses regarding growth regulation by morphogen gradients. This model does not take into account that the shape of the concentration gradient decreases exponentially and not linearly. Thus, the simplest calculation based on the shape of the morphogen concentration profile that would lead to a uniform response by cells would be to divide the slope of the gradient by the magnitude of the gradient at the cell’s location.

More recently, Wartlick et al. measured DPP in discs at multiple time points during development [56]. They observed that the amplitude of morphogen gradient increases with disc size. Based on correlations with DPP levels and disc size, they propose a model which states that cells divide when the magnitude of DPP increases by a factor of about 50% in the cell. A computational study of the consequences of DPP concentration levels regulating cell division suggests that such an instructive regulatory mode may explain the scaling properties of the tissue during early stages of growth [68]. The increase of DPP levels begins to slow at the end of the developmental period leading to longer cell cycle periods. Growth eventually terminates when the cell cycle becomes too long. This growth termination model provides an alternative or complementary mechanism that does not require mechanical feedback. An alternate model proposed by Schwank et al. is based on genetic experiments, showing that wing discs still grow upon removal of DPP and an important downstream target Brinker (BRK) [54]. These results suggest a more permissive role in DPP regulating size control. While this permissive model can also explain the relative uniformity of growth in the wing disc, a mechanism terminating growth, such as that suggested by the mechanical feedback

hypothesis, thus becomes necessary (for a more detailed account on the different morphogen growth models see [30]).

Resolution of whether morphogens are instructive or permissive for growth is still required. In particular, there is a need to define the cellular input/output functions that convert a dynamic morphogen signal into a uniform growth profile seen at later stages of development. Here, *in silico* models of morphogen signaling that adapt to tissue growth have the potential in guiding experiments for differentiating between model scenarios [69]. One result may be the realization that morphogen-dependent growth may be both instructive and permissive depending on the developmental stage of growth. Growth regulation may differ between the early and rapid stages of growth and the terminal, slower phase that leads to growth termination.

3. The mechanical feedback hypothesis

3.1 Initial formulation

In addition to morphogens and growth factors, mechanical tension has also been shown to promote proliferation in many mammalian tissues such as skin cells and is recognized as an important factor in plant growth (see, amongst others, [70–73]). Geometric constraints and extent of spreading can instruct cells to either grow or undergo apoptosis [16,74]. Mechanical cues also instruct cellular processes such as gene expression in *Drosophila* embryogenesis [75]. An early exploration of mechanical feedback in wing disc growth by Shraiman considered the mechanical effects of differential growth rates in lineage-related groups of cells (here and elsewhere called clones) that grow faster than surrounding cells (for an overview of modeling techniques implementing the mechanical feedback hypothesis, see Table 1 and Figure 2). In this scenario, cell populations manifesting a faster growth rate compared to their neighbors would be expected to grow exponentially [76]. Outside the clonal boundary, stretching of surrounding cells is predicted to increase growth rates. The model assumes insignificant rearrangements of cell neighbors on the timescale of cell divisions, thus allowing the tissue to be approximated as a two-dimensional elastic solid with a defined shear-rigidity modulus. The main prediction of this “integral-feedback” model is that a faster growing clone will build up compression dependent on the integral of the differences in growth rates between the two populations of cells. This negative feedback would tend to slow growth compared to what would be observed in the absence of mechanical feedback. Consequently, one experimentally verifiable prediction is to quantify the difference in average clonal size between wild-type (WT) clones and fast growing mutant clones over time, which has not yet been done systematically. This ratio should decrease over longer time scales if mechanical feedback is significant in the system.

Allowing cells to rearrange the edges that they share with their neighbors introduces a relaxation term that relieves the build up of pressure due to differences in growth rates between cells. While some rearrangements are observed when wing discs are cultured in a growth medium optimized for organ culture [77] and rearrangements have also been observed *in vivo* [64,78], the number of rearrangements is not sufficient to fully relax stress within the tissue [64,78]. Additionally, the finite thickness of epithelial layers introduces a smoothing out of pressure gradients on the length-scale of cells resulting in non-autonomous

compressive effects on cells neighboring fast growing mutant clones. Higher levels of compression may lead to increased levels of apoptosis at clonal boundaries as is seen in the case for cell competition [79]. This model was based on a continuum approach, which did not include a description of individual cell-cell mechanics. However, a recent quantitative study accompanying a simple computational growth model provides evidence that the increased apoptosis due to cell competition has negligible impacts to the increased size of winner cell clones (those that grow more quickly) [28].

Mechanical feedback has also been hypothesized to modulate morphogen-induced proliferation [80,81]. In a second tissue-scale, continuum-level model, Aegerter-Wilmsen and colleagues investigated the interplay between morphogens and mechanical forces ("morphogen + mechanical stress"). This phenomenological model postulates that growth is stimulated by high levels of morphogen signaling at the center of the disc but inhibited by increasing compression above a hypothetical threshold. Mechanical tension at the periphery induces growth even though morphogen levels are lower [81]. The phenomenological model approximates the wing pouch to be a radially symmetrical, two-dimensional elastic sheet with constant cell density. This assumption was justified as a first order approximation by observations of the connections of the cytoskeletons between neighboring epithelial cells. As in the "integral-feedback model" [76], tension was not allowed to relax due to cell sorting, and apoptosis was not included, due to the low levels of cell death observed in wild-type growth [82]. The "morphogen + mechanical tension" model simulations predict growth only in the very center of the disc during initial growth and can explain observed position-dependent growth rates for clones with altered levels of DPP morphogen signaling and the non-homogenous growth observed with uniform DPP signaling across the pouch [50,83].

The studies discussed above developed continuum descriptions of growing tissues. To recapitulate the connectivity (topology) and geometry of individual cells, vertex models can be used to approximate the shapes of epithelial tissues (reviewed recently by [84]). In vertex models, cells are modeled as 2D polygons with nodes (vertices) connected by edges. This assumes that most of the forces between cells are concentrated near the apical surface where E-Cadherin is located to provide adhesion between cells along the edges and to organize the contractile actin-myosin around the apical cortex.

In a contemporaneous model to [81] presented by Hufnagel et al. [80], cells were approximated by polygons that grow at a rate that depends on both the morphogen concentration and compression. Growth is induced when cells sense a morphogen concentration that exceeds a threshold while mechanical compression inhibits growth. Compression is computed based on the minimization of the total energy in the system, which occurs when the volume of a cell is close to a hypothetical "target volume" and its perimeter and height variances among its neighbors are minimized. Cells were chosen at random to divide with a probability proportional to their growth rate. Based on these rules, the simulation predicts spatially uniform proliferation rates. Growth terminates once the compressive stress in the center of the pouch is sufficient to counteract morphogen-induced growth. At the periphery of the tissue, growth stops when cells spread past the spatial distance where the local morphogen concentration is sufficient to induce growth. Mechanical

feedback thus “homogenizes” growth across the wing disc. Similar to the conclusion from the Aegerter-Wilmsen et al. model, the model proposed by Hufnagel et al. is able to recapitulate uniform proliferation throughout the disc. It also predicts that variation in the final size between multiple simulations (representing a set of biological samples) is less than during earlier time points. Experimentally, they measured the shape of the DPP gradient during the last 48 hours of development and did not observe a statistically significant change in the DPP length scale justifying their use of a static morphogen gradient. Later attempts at quantifying DPP signaling showed that the shape of DPP gradient proportionally scales or adjusts to the dimensions of the wing disc during earlier stages development (the earlier exponential phase of growth) [56,85]. This assumption of a static morphogen gradient, while potentially operative during the final hours of growth, may suggest that mechanical feedback does not play a significant role during the exponential phase of growth and may not be the determining factor for growth termination [49].

3.2 Cell topology

The progressively refined mechanical models showed that mechanical stresses might play an important role in modulating morphogen-induced growth and prompted many specific questions about the role of mechanical stresses at the scale of individual cells within the tissue. For example, how is cell packing affected? As the apical surface of epithelial cells contains the highest concentration of actomysoin at the cortex, most of the internally generated forces in the cell are concentrated there. The question of cellular topology is an active area of research, studied using computational and biomechanical approached by several groups from multiple angles (reviewed in [86,87]). The work of Gibson and colleagues simulated a network of cells using a Markov Model – a stochastic model that is updated based solely on the current state - to predict the distribution of polygon classes which were then compared to the observed distribution of polygons in the wing disc [88]. The model argues that the distribution of polygons observed experimentally can be reproduced by a model that assumes uniform cell division but does not explicitly include mechanical forces.

However, cell topology depends on mechanical interactions between members, and mechanisms of many observed features of cell topology require including mechanical feedback in the model [89]. Farhadifar and colleagues used an experimentally measured distribution of cell polygon class to constrain a vertex-based model governed by an energy equation. Their model was further validated by laser ablation of the membranes connecting neighboring cells (edges) to measure tension between neighboring cells [90]. The energy of the system is given by

$$E = \sum_{\alpha} \frac{K_{\alpha}}{2}\left(A_{\alpha} - A_{\alpha}^{(0)}\right)^2 + \sum_{<i,j>} \Lambda_{ij} l_{ij} + \sum_{\alpha} \frac{\Gamma_{\alpha}}{2} L_{\alpha}^2$$

which takes into account terms for a target cell area, $A^{(0)}$. l_{ij}, is the junction length, between nodes i and j, and L_{α} is a cell perimeter (Fig. 2A). Cells were selected at random to grow and divide. A search was performed to find the range of parameters for which simulations

were able to reproduce results in agreement with the experimental data relating to the polygon distribution and cell areas.

The vertex model by Farhadifar et al. [90] was extended to investigate the role of mechanical stresses on cell topology [91]. Four scenarios were modeled: with and without cell rearrangements and with and without mechanical stress. The effects of mechanical stress were implemented through cell area dependent division rates. In each scenario, polygon distributions as well as mitotic clustering were computed for both mitotic cells and all cells in the population, and compared to experimental observations. It was found that only the simulations that included mechanical stress dependent divisions were able to replicate the experimental distributions.

These models showed that growth inhibited by compression could explain the uniform proliferation resulting from a non-uniform gradient, while reproducing key aspects of cell topology. In all of these models, the connection between mechanical stresses and growth and division is hypothetical. How exactly does compression result in growth regulation? To answer this question, Aegerter-Wilmsen and colleagues extended their model to include signaling pathways that explicitly connected mechanical forces to growth regulation [92] (Figure 2). Hypothetical interactions determined for the proteins connecting mechanical forces (Armadillo, the Drosophila version of β-catenin, the Hippo pathway transcription factor Yorkie, and Dachsous). All other protein activities and interactions are determined from previous experiments. This model was able to reproduce many experimental findings related to the disc size regulation, including consistent growth rate curves, spatially uniform growth, non-autonomous growth generation by clones, among other findings directly related to the protein interactions.

3.3 Experimental validation of the mechanical feedback hypothesis

One of the challenges in using mathematical models to better understand the paradox of uniform growth in the wing disc is the difficulty of designing experiments to test the predictions of these models in a meaningful way. Experimental validation of the mechanical feedback hypothesis is still largely circumstantial and correlative to a significant degree due to the technical challenges of applying mechanical forces within growing, moving animals without elucidating general stress responses. Approaches to date have relied largely on either testing correlative predictions based on theoretical models or developing *in vitro* approaches to apply mechanical forces to the organ.

3.3.1 Measuring mechanical properties in organs—A basic assumption in mechanical feedback models is the buildup of compression in the center of the wing pouch. To verify this assumption, Aegerter and co-workers measured changes in birefringence in the wing discs to provide an indirect measure of mechanical stress in the tissue [93]. Application of mechanical forces on birefrigent molecules leads to changes in the degree of retardance of light passing through the material. Their measurements of retardance are consistent with compression that builds in the center of the disc during development. In follow-up measurements of photoelasticity in the wing disc that was accompanied by

physical stretching, the Young's modulus was estimated to be on the order of 10^5 with the wing disc showing highly elastic behavior [94].

While limited to short periods of time for a given imaging session, wing discs can be imaged at cellular resolution *in situ* [95,96] over the development of individual larvae. In the first recent study which relied on compression of larvae for immobilization, several observations were made: individual growth curves are highly variable while final size is less variable, and a muscle fiber attached to the wing disc as well as other filaments appears to apply substantial tension to the whole disc. When this fiber is cut by dissection, relaxation and a reduction of the apical size of wing disc cells is observed. It will be interesting to test how targeted ablation of this muscle fiber affects organ growth and patterning in the wing disc. Further, possible implications of extrinsic of forces by muscle fibers application to wing disc growth have not been incorporated into mechanical models to date. A more recent effort avoids the complication of external compression by using an anesthetic to immobilize larvae for brief intervals, followed by recovery [96]. This approach suggests that imaging compressed larvae can lead to artifacts. Systematic application of *in vivo* imaging to the growth and signaling dynamics of individual cells over multiple time points may help resolve our understanding of how morphogens and mechanical forces jointly regulate tissue growth at different stages of development.

The forces on cellular junctions are typically measured by laser ablation of cell edges. Recent work has been used to estimate relative tensions at the cellular level by inferring stresses from cell shape and orientation from confocal micrographs of the apical surfaces of cells using inverse methods [97,98]. These approaches offer the potential of acquiring relative tensions and pressures of cells from images.

3.3.2 Manipulating mechanical stress in epithelia—Direct mechanical stretching of wing discs presents some technical challenges since the organ is both small and extremely fragile. However, based on a bioassay culturing protocol to measure mitotic rates (described in [77]), short term (1 hr.) stretching of *ex vivo* cultured discs showed an increase in observed number of mitotic cells [99], as would be expected if mechanical tension regulates the cell cycle. However, some caution needs to be applied as these initial promising results have inherent limitations: the short culture time enabled only analysis of mitoses within the wing disc, and the stretching apparatus relies on a very stressful method of adhering large regions of the wing disc to glass slides that are mechanically shifted in relation to each other. Ideally, in vivo data would be optimal, but long term, high-resolution imaging of growing, moving larvae, while not impossible, is technically challenging. Nonetheless, these data provide a first direct test of the mechanical feedback hypothesis in *Drosophila* wing discs.

Recently, Hufnagel and colleagues directly tested the effect of spatial and mechanical constraints on a mammalian models of two-dimensional epithelia (Madin-Darby canine kidney-2, MDCK-2 cells, chosen in part because multiple rounds of the cell cycle can be imaged in long-term culture experiments) by either removing a physical constraint for cells or by stretching the underlying substrate [100]. They demonstrate cross-sectional area dependence in the G1-S transition probability. If cells are too crowded then the probability of entering the cell cycle decreases. The fraction of cells entering the cell

cycle increases upon stretching and decreases upon relaxation, without showing a hysteresis effect. Furthermore, this cell cycle dependence on mechanical stretching is dependent upon ERK signaling. In this study, a one-dimensional model of stretching predicts that wound healing or extensive cell migration depends upon cells entering the cell cycle. They then validate this prediction by blocking ERK signaling with a MEK inhibitor and show that cell migration is halted after an initial period of expansion. In the future, it will be interesting to test if these observations in two-dimensional MDCK cells apply equally to the organ context of the wing imaginal disc, potentially strengthening the case for an integral role of mechanical feedback in organ size regulation. It will be especially interesting to evaluate which signaling pathways are most responsible for mediating a putative G1-S block due to mechanical constraints in the wing disc. ERK and Hippo signaling are likely chief suspects for initial investigation [101].

Recently two studies have quantitatively mapped the strains and stresses in the wing disc during development [64,78]. In the study by LeGoff et al., image analysis was performed to investigate cell deformations as well as systematic probing of tension at cell membranes using laser ablation to measure the retraction speed of the cut membrane. They found that there is indeed higher tension away from the geometric center of the wing pouch. It was also found that cells polarize their acto-myosin cortex in response to the mechanical forces found in the wing disc. The Fat/Dachsous planar polarity pathway did not seem to be chiefly responsible for the anisotropy in tension across the wing pouch. Interestingly, loss of actomyosin contractility through pharmacological inhibition did not abolish the tensions in the pouch, suggesting that Myosin II polarization is a response to mechanical forces rather than a cause. In both [64,78], hyperplastic clones are under compression and cause non-cell autonomous stretching of neighboring cells as described theoretically by Shraiman [76]. Work by Gibson and colleagues found that cell shape and mechanical tension tends to orient cell division through orientation of the mitotic spindle so that cell division tends to be parallel to the direction of higher tension. This provides a mechanism to relieve stress within the tissue [102].

4. Conclusions and open questions

On one level, significant supportive evidence for the mechanical regulation of growth has been accumulating. Yet, the mystery of organ size control still remains to be fully solved. Much can be learned by comparing studies of other model systems such as in plants (reviewed in [103]) and in the mammalian limb bud (reviewed in [15]). Even when genes are not conserved, network topology and general mechanisms may emerge leading to general heuristic rules about the roles of mechanotransduction signaling in organogenesis.

Exploration into how mechanical feedback regulates organ growth has been most extensively investigated in wing imaginal discs, yet much is still unknown regarding how mechanotransduction pathways cross-talk with morphogen signaling cascades. Most work in the wing disc has focused on the 2D apical surface. Stereotypical buckling occurs which may relieve compression and delay growth arrest as postulated in [80]. However, neither the mechanics of buckling, nor the effects of buckling on patterning have been explored computationally. Nor, has the role of the peripodial membrane been considered

in computational models [66]. To further validate the mechanical feedback hypothesis, existing criticisms of the model will need to be addressed. In particular, it is not clear that the compression that occurs during physiological growth is sufficient to explain growth termination [49]. As our understanding of the wing disc development grows through the application of in vivo longitudinal studies [96], models will need to be updated and revised.

Progress toward answering the large biological mysterious such as size control always leads to even more questions. Some of these include:

1. As the tissue is stretched, either externally or internally through growth, is there feedback into morphogen gradient formation such as changing the kinetics of morphogen transport and internalization?
2. Are mechanisms of mechanical feedback on growth in the wing disc conserved in other contexts such as plant growth and mammalian development?
3. What are the mechanotransduction pathways that mediate regulation of the G1/S and G2/M checkpoints?
4. Is the stereotypic buckling in the wing disc largely determined genetically [104] and how does buckling feedback into wing disc growth and patterning?
5. Does mechanical feedback play a central role in organ growth control or are there other factors that need to be considered?

Vertex models, the most frequent approach towards modeling the wing disc, can be applied to buckling as has been done for modeling dorsal appendage formation [105]. Hyperplastic mutants that do not show growth termination still face mechanical constraints (Fig. 1D). It is unknown how growth patterns are affected in hyperplastic mutants, and computational modeling studies in different genetic backgrounds are generally lacking.

Beyond internally-generated mechanical feedback, developing models that explicitly integrate both intrinsic, genetic factors with extrinsic signals such as hormones and putative mechanical constraints are expected to provide a deeper understanding of how size is regulated during development and in disease conditions. Beyond fundamental questions regarding the size of animal organs, the interplay between mechanical forces and cellular proliferation is likely to find applications in bioengineering. For example, lessons learned on scaling, tissue biomechanics and cellular function will help guide the design and scaling analysis of organ modules in organ-on-a-chip applications [106–108]. Clearly, the story of the mechanical feedback hypothesis for organ growth control is still just beginning, with many subplots waiting to be more fully pursued.

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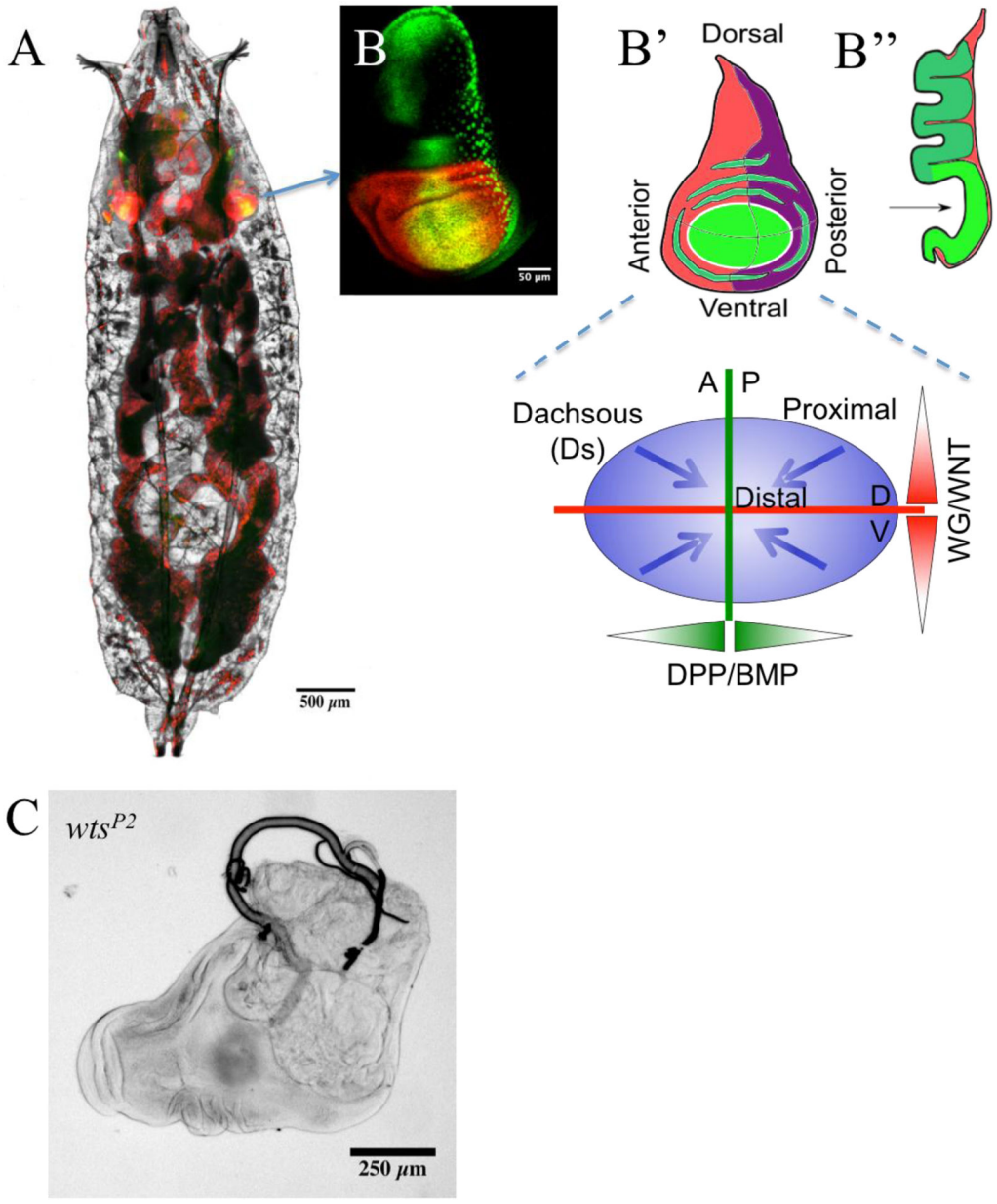

Figure 1:

(A-B). The wing imaginal disc as a model system for studying organ growth control. The wing discs are epithelial sacs loosely attached to the tracheal system during larval growth (A). During the third instar larvae (shown in B) the wing disc assumes a folded morphology with the pouch cells prominently forming an ellipse shape in the center of the "pear"-shaped organ (B' and B"). Red marks the *nubbin* expression domain (*nubbin-mcherry*) and green marks *dad* expression (*dad-GFP*), which a target and inhibitor of DPP signaling. The transgenic fly line was a gift from the Affolter lab. During metamorphosis, the pouch will undergo eversion and elongation to form the adult wing. The pouch is patterned by morphogen gradients that establish a Cartesian coordinate system along the anterior-posterior (AP) and dorsal-ventral (DV) axes. The Dachsous-Fat pathway forms a

gradient along the proximal – distal axis. C: Hyperplastic mutants such as wts^{P2} do not terminate growth and become highly folded. D: Proliferation depends on the milieu as shown

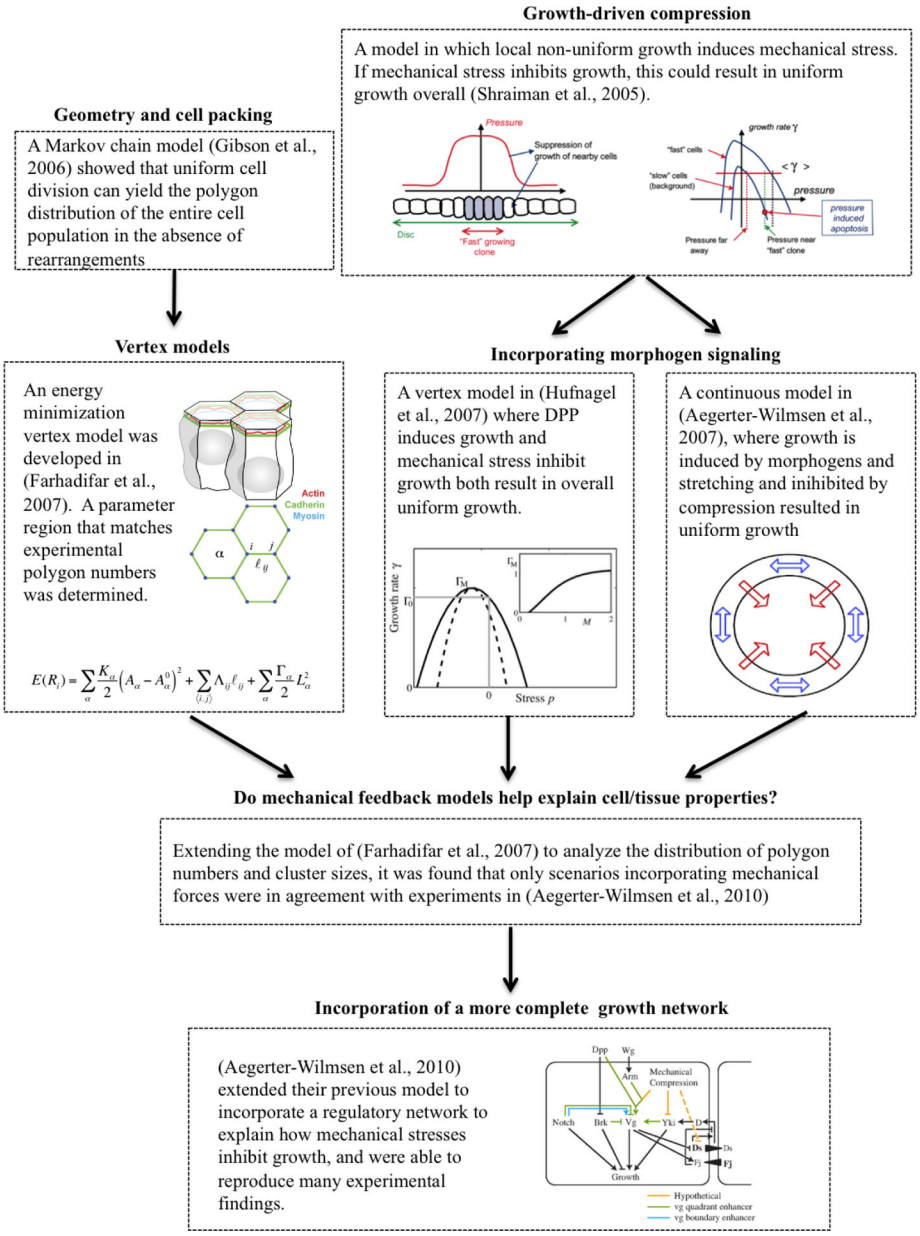

Figure 2:

Graphical representation of selected works exploring the mechanical feedback hypothesis in wing discs. Inset figures are adapted from [76,80,81,88,90–92]. Used with permission.

Table 1:

Brief, selected overview of the models that have investigated size control in the wing imaginal disc [76,80,81,88,90–92].

Model Type	Contribution
Continuous	The early models incorporating mechanical feedback were continuous models, which are typically used to model the entire wing disc. (Shraiman et al., 2005) approximates cells as a 2D elastic solid and models the effects of mechanical stress resulting from non-uniform local growth rates. (Aegerter-Wilmsen et al., 2007) models growth of the wing disc, which is induced by the combination of morphogens and stretching and inhibited by compression.
Vertex	More detailed vertex models are used to model individual cells, whereas continuous models typically model an entire region of cells. A vertex model incorporating mechanical feedback was developed in (Hufnagel et al., 2007) to investigate uniform growth resulting from a non-uniform morphogen gradient. Later, the vertex model of (Farhadifar et al., 2007) was extended to include mechanical feedback by division rates that depended on cell areas (Aegerter-Wilmsen et al., 2010).
Hybrid	To explain how mechanical feedback could regulate growth rates, (Aegerter-Wilmsen et al., 2010) was extended to include a regulatory network based on known protein interactions as well as hypothetical interactions for the interactions resulting from mechanical stresses (Aegerter-Wilmsen et al., 2010).