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Forward and feedback control mechanisms of developmental tissue growth

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Abstract

The size and proportions of animals are tightly controlled during development. How this is achieved remains poorly understood. The control of organ size entails coupling of cellular growth and cell division on one hand, and the measure of organ size on the other. In this review we focus on three layers of growth control consisting of genetic patterning, notably chemical gradients, mechanics and energetics which are complemented by a systemic control unit that modulates growth in response to the nutritional conditions and coordinates growth between different organs so as to maintain proportions. Growth factors, often present as concentration dependent chemical gradients, are positive inducers of cellular growth that may be considered as deterministic cues, hence acting as organ-intrinsic controllers of growth. However, the exponential growth dynamics in many developing tissues necessitate more stringent growth control in the form of negative feedbacks. Feedbacks endow biological systems with the capacity to quickly respond to perturbations and to correct the growth trajectory to avoid overgrowth. We propose to integrate chemical, mechanical and energetic control over cellular growth in a framework that emphasizes the self-organizing properties of organ-autonomous growth control in conjunction with systemic organ non-autonomous feedback on growth.

Introduction

The range of body sizes in animals varies by nearly six orders of magnitude from the smallest known insect *Dicopomorpha echmepterygis* measuring only ~140µm (indeed, smaller than single celled ciliates such as *Paramecium*), to the blue whale, the largest mammal measuring over 20 meters. Despite these vastly different dimensions all animal species precisely control their size. Two basic facts manifest tight

1 control over organism, organ and tissue size. Within a species and in defined environmental (i.e.
2 nutritional) conditions, the size of organisms is very reproducible. Moreover, organisms are largely
3 symmetrical and the size of different organs scale with respect to each other so that functional proportions
4 are ensured [1].

5 Although organisms control their final size in the adult stages, growth is also highly controlled during
6 development. Situations are very different depending on organisms. In oviparous organisms, such as
7 insects, amphibians, reptiles, birds and fishes, the size of the hatching animal is limited by the size of the
8 egg shell. In insects such as *Drosophila*, the growth phase which determines the size of the adult is
9 restricted to the larval stages. In contrast, mammalian embryos grow *in utero*, which limits the size of the
10 developing baby, followed by a major growth phase until growth termination at the end of puberty.

11 In this review we address the mechanisms of developmental growth. Both older and more recent evidence
12 in amphibians and in *Drosophila*, indicated that the control of organ size partially rests on mechanisms
13 that are autonomous to these developing structures, revealing mechanisms for organ-specific size
14 measurement. For instance, heteroplastic eye or limb grafts between large and a small species of
15 salamanders showed that grafts grow to a final size that is set by their own genotype (species) and not by
16 the nature of the host [2]. Likewise *Drosophila* imaginal discs, the precursors of adult appendages, from
17 late larvae transplanted in the growth permissive environment of adult females grow to the normal final
18 size they reach in the larva [3]. These data illustrated that growing organs are endowed with the capacity
19 to 'read' their size and led to the search for mechanisms underlying local organ-size measurement.
20 Fundamentally, any biological parameter whose value is a proxy for tissue size could be used as a 'ruler'
21 to control tissue growth. Importantly, size information obtained by organ intrinsic rulers is modulated by
22 organ extrinsic signals that adjust growth to the given environmental and nutrient conditions (in the
23 scope of this review we will not discuss the details of the systemic nutrient sensing system and refer the
24 reader to several excellent reviews [1,4]). Nutrient sensing systems like the Target of Rapamycin (TOR)
25 network [5] and input from the insulin/insulin-like growth factor pathway connect the nutritional state of
26 the animal with cellular and tissue growth. Hence, organ intrinsic information is complemented and
27 modulated by organ extrinsic, systemic cues to ensure that organs obtain dimensions that are of
28 proportionate size to the animal size. In imaginal discs growth is initially exponential and eventually
29 arrests. Size control thus entails control over growth arrest.

30 The fundamental unit of tissue and organ growth is the cell. Tissue growth is therefore the result of two
31 inherently linked processes: cellular growth (mass and volume increase) and cell number increase by cell
32 division. By definition cellular growth entails mass and coordinated volume increase while divisions
33 control cell number and density. Cell density and hence the average cell size in a given cell type is very

1 precisely controlled[6,7]. Cell mass and volume increase occur during the cell cycle and is tightly linked
2 to the cell cycle progression such that, within a given cell type in a multicellular organism, cell size
3 variance is very limited [6,8,9]. Consequently, tissue growth and arrest require a mechanistic coupling
4 between cell growth and cell cycle progression [10] on one hand and some direct or indirect measure of
5 tissue size on the other. In essence, cells need to compute local and non-local information so they can
6 decide when they should arrest growth and become quiescent. Recent advances in the study of both
7 prokaryotes and eukaryotes have illuminated mechanisms underlying cell growth and the links between
8 cellular growth and cell division (see excellent reviews [8,9,11,12]). However, how such cellular
9 processes are controlled by non-local mechanisms of tissue or organ-scale measurement remain more
10 uncertain.

11 Here, we review three main modes of regulation of tissue growth within an organism, namely control
12 based on genetic and coupled biochemical processes, mechanical control and, finally, energetic control
13 that encompasses metabolic processes. We advocate the need to integrate these three modes of regulation
14 (biochemical, mechanical and energetic). We also discuss how forward and feedback control mechanisms
15 governing biochemical signaling, tissue mechanics and bioenergetics drive sustained cell growth and cell
16 division and finally yield growth arrest at the end of the development.

17 One way to set up information on the current size of the tissue is to use a molecular ‘ruler’, such as a
18 chemical morphogen gradient that defines a length scale such as positional information within a tissue.
19 Such a molecular ruler operating at the tissue scale may be a genetically determined pre-pattern that
20 controls hierarchically cell growth and division in a deterministic and forward manner. In this view, cell
21 growth and proliferation could be programmed and thus patterned in space and time by the complex
22 network of positional information laid down in the growing and developing organ. However, two types of
23 evidence argue against a simple feed-forward, deterministic program that controls tissue size and argues
24 for the need for feedback mechanisms that endow the growing system with regulative, that is to say self-
25 organized tissue size control. First, tissues can reach their target size despite significant perturbations in
26 the spatial or temporal pattern of cell growth and proliferation, or cell survival as illustrated in *Drosophila*
27 wing imaginal discs [13–16]. Mosaic perturbations of cellular growth rate or cell proliferation do not
28 affect the shape nor the size of the wing disc and the final adult wing. Thus, many different patterns of cell
29 growth and different “growth trajectories” give rise to similar if not identical final tissue size. Thus, tissue
30 size requires information at the tissue scale independent of microscopic details of cellular growth. Second,
31 growth is exponential in many tissues, until it becomes linear and finally arrests. In an exponentially
32 growing tissue, the observed precision over final tissue size would require extraordinary precision over the
33 decay of the growth rate until final size is reached. A minute variation in the growth rate would give rise

1 to a vastly different final size. This calls for built-in feedback mechanisms. One might refer to such
2 feedback-based control of growth as ‘self-organized’, reflecting the capacity of complex systems to create
3 order in space and time as a result of local collective interactions [17].

4 The review will now address the three modes of control (biochemical, mechanical and energetics) and
5 emphasize forward and feedback control mechanisms.

7 **Biochemical control of growth**

8 Morphogen gradients have long been in the spot light as molecular rulers that can provide size information
9 required for organ intrinsic size control. As secreted signaling factors, morphogens form concentration
10 gradients within the target tissue surrounding their domain of expression [18–20]. These concentration
11 gradients are sensed and interpreted by cells in the target tissue quantitatively, inducing different
12 transcriptional responses depending on the morphogen concentration sensed and therefore on the distance
13 from the morphogen source (Fig.1A). Thereby morphogen gradients can pattern a target tissue and
14 provide positional information to cells in a variety of systems [19,21,22]. Furthermore, the molecular
15 properties of morphogen production, dispersal in the tissue and signaling dynamics could provide a
16 biological length scale set by the range of the morphogen gradient that could instruct size information.

17 One of the showcase example of morphogen-mediated growth control remains the Decapentaplegic (Dpp)
18 [23–25] gradient during the development of the *Drosophila* wing imaginal disc, the larval precursor of the
19 fly wing. Dpp is expressed in a central stripe in the wing disc from where it forms a concentration gradient
20 into the adjacent target tissue [26,27]. The Dpp gradient has been extensively studied for its function in
21 patterning the wing disc [23–25], while its function in growth control remains controversial[18,28]. Dpp is
22 clearly crucial for disc growth since overexpression of Dpp increases wing size while mutations that
23 reduce levels strongly impair growth and reduce wing size [29]. The developmental duration of expression
24 of Dpp also increases tissue size [30]. Importantly, several studies highlighted the importance of the Dpp
25 gradient for growth [30–32]. Furthermore, recent evidence suggests that the Dpp morphogen gradient
26 promotes anisotropic growth along the anterior/posterior axis of the wing disc while the Wingless
27 morphogen which is expressed in an orthogonal domain promotes growth along the orthogonal axis [33].
28 In many cases, cell proliferation has been used as a proxy for growth, while the actual growth dynamics
29 (volume and biomass increase) of the wing disc remain vaguely studied. Different models have been
30 proposed to explain size control by the Dpp morphogen, namely more generally how graded levels of a
31 signaling molecule can result in both the uniform proliferation pattern observed in the wing disc [34] (Fig.
32 1B) and the final wing size [35].

A first type of model proposed to evaluate the spatial differences in Dpp levels between neighboring cells. The '*Linear Slope Model*' (Fig.1C) suggested that cells read the steepness of the concentration gradient ($\Delta c/\Delta x$) [36]. Assuming a linear Dpp gradient this model can explain uniformity of proliferation, since $\Delta c/\Delta x$ is uniform for a linear gradient at a given time point. Also, the model can explain growth termination assuming that the gradient scales with tissue size and thereby flattens with increasing tissue size and growth halts once the gradient becomes too shallow. Given the exponential shape of the Dpp gradient [37] a modified model termed the '*Exponential Decay Slope Model*' [28,35] was proposed (Fig.1D). When cells compute *relative* spatial differences in Dpp levels (the slope normalized by concentration or $(\Delta c/\Delta x) \cdot 1/c$) the readout becomes position-independent and can explain spatial uniform proliferation. However, uniform expression of an activated form of the Dpp receptor Thickveins in the central part of the wing disc does not alter disc growth [38]. This suggests that disc growth does not strictly depend on graded Dpp levels but uniform Dpp signaling is sufficient for disc growth, contradicting this class of models.

Instead of reading spatial differences, cells could compute temporal changes in Dpp levels as proposed by the '*Temporal Dynamics Model*' [37] (Fig.1E). This model proposes that cell division is set by the relative temporal variation in Dpp concentration ($\Delta c/\Delta t \cdot 1/c$). In the developing wing it was proposed that cell division occurs upon a 40% increase in cellular Dpp signaling. Indeed, experimental results using a Dpp::eGFP reporter suggest that Dpp source levels increase during disc growth following a power law. In addition, the Dpp gradient shape follows an exponential decay function and its length adjusts/scales to tissue size. These two findings imply that the relative shape of the gradient does not change during development (when normalized to tissue width (L) and to maximum Dpp levels), and therefore the relative temporal increase in Dpp levels ($\Delta c/c(t)$) is uniform in space (Fig.1E, bottom). The model elegantly provides a mechanism for uniform proliferation by a graded input signal and more distantly provides an explanation for growth termination. Though the model hinges on a key phenomenological law, that is yet unaccounted for, namely that the amplitude of the Dpp gradient follows a power law relationship with tissue size. Furthermore, as such this law couples growth and Dpp concentration increase but the causal relationships may be more complex than a simple forward control mechanism: the gradient of chemical species regulates growth and growth changes the chemical pattern by advection and diffusion [39]. Despite providing an elegant feedback mechanism several observations do not seem to fit this model. In particular, removing Dpp and its antagonist Brinker, a major repressor of growth [40], does not block tissue growth [38] and gives rise to nearly normal wing size [30], arguing that a Dpp gradient may not be essential for tissue growth per se. It will be important to directly assess the dynamics Dpp signaling at the single cell level and to decipher the origin of the power law function between Dpp concentration and tissue size.

A distinct type of model considers the distribution of absolute Dpp levels. While the previous two models propose an instructive function of Dpp, the '*Growth equalization Model*' (GEM) [24] postulates a potential permissive role of Dpp signaling for growth (Fig.1F). In *dpp/brinker* double mutants the proliferation rates are increased in the periphery and decreased in the center, suggesting that the growth potential in the wing disc tissue is non-uniform. It was postulated that an inherent non-homogenous growth potential (lower in the center and higher in the periphery) is equilibrated by Dpp-dependent repression of Brk in the center of the disc. Thereby, the lower central growth potential is increased by removal of Brk and the higher peripheral growth potential is reduced by the presence of Brk, equalizing the growth potential along the disc tissue leading to uniform proliferation (Fig.1F, bottom). Such a model would promote a view where Dpp's role is restricted to the central region of the pouch where Dpp signaling activity is repressing Brk, while the role of Dpp in the periphery remains unclear. However, a study by the Milan lab investigates clonal growth in the center versus the periphery upon knock-down of Dpp [30]. The study observes reduced growth in the periphery in the absence of Dpp, and suggests a role for Dpp in the peripheral region. However, the study does not establish whether the requirement of Dpp is a direct one. A possible alternative interpretation is that the knock-down of Dpp results in changed in tissue mechanics and peripheral cells reduce their proliferation due to decreased mechanical stretching (see next section)". Despite numerous investigations it remains poorly understood how morphogen signaling is molecularly linked to growth control. There is evidence that repression of the growth regulator Myc by Brk is linking Dpp activity to growth control [41]. Also Dpp has been proposed to act in conjunction with Wingless in a feed-forward loop to recruit cell to the growing wing pouch in a Yorkie/Hippo dependent manner [42]. We have seen that morphogen gradients have per se the capacity to provide biological length scale. While there is evidence that indeed their range is relevant for growth and size determination [42] recent results indicate that Dpp signaling might not be involved in controlling growth termination [43]. Despite these unresolved questions, none of the above discussed models accounts fully for growth control in organs. They rather provide mechanisms on how graded Dpp levels can instruct uniform proliferation and growth. Increasing evidence suggests that uniform low levels of Dpp are sufficient to sustain growth [30,32], proposing a permissive role of Dpp in growth control. The actual modes of morphogen dispersal remain debated, but recent synthetic approaches reveal that diffusion, surface trapping by signaling receptors and facilitated diffusion by non-signaling receptors account for proper long range Dpp spread, tissue patterning and tissue size [44]. Also the phenomenon of gradient scaling provides evidence that feedback loops operate, since scaling requires that the effective diffusion coefficient and/or degradation constant scale with tissue size, such as in the expander model [45]. Pentagon (Pent) [45,46], a diffusible protein acts as an expander of the Dpp gradient but is at the same time repressed by Dpp signaling via negative feedback loop. However, the role of Pent as a feedback

1 expander has been challenged recently and an alternative hypothesis was proposed in which morphogen
2 signaling regulates the levels of receptors and co-receptors via multiple feedback-loops to archive robust
3 scaling of the Dpp gradient [47].

5 **Mechanical control of growth**

6 Tissue mechanics could also provide a mechanism for the regulation of tissue growth and tissue size.
7 Evidence from *in vitro* experiments clearly establish that cell proliferation is influenced by the mechanical
8 environment. For example, increasing substrate adhesion of cultured cells results in cell flattening and
9 stretching that induces cell proliferation via YAP-TAZ [48]. Also, active stretching of cultured MDCK
10 cells results in an induction of cell proliferation which is dependent on a mechanosensitive transmembrane
11 receptor Piezo I [49]. Stretch-induced activation of Piezo I results in calcium signaling and downstream
12 activation of Cyclin B. In contrast, mechanical compression results in cell extrusion[50]. The concept of
13 homeostatic pressure was proposed on theoretical grounds to capture the idea that cells define a
14 mechanical threshold below which cells grow and divide, and above which cells die. Distinct cell
15 populations may genetically tune their homeostatic pressure [51]. In a thought-experiment, 3D cultured
16 cells are confined in a container that is permeable to everything but cells, cells grow against a wall resisted
17 by a spring until the culture reaches the homeostatic pressure at which apoptosis and cell division balance
18 each other. Certain tissues (e.g. cancerous tissues) may sustain higher internal pressures and outcompete
19 “weaker” (i.e. non-cancerous) tissues by increasing their homeostatic pressure [51].

20 The role of mechanics on cell proliferation and on final size control was investigated in the *Drosophila*
21 wing disc. During wing disc growth, cell shape and surface area become non-uniform (Fig.2A). A radial
22 gradient of cell surface area forms, with smaller cells in the center and larger cells in the periphery.
23 Concomitantly, cells in the periphery are elongated in tangential direction. Laser cutting experiments
24 showed that peripheral cells are under tension in the tangential direction [52,53].

25 A mechanical feedback model was proposed [54] whereby 1) the cell growth rate depends on pressure and
26 reaches a maximum value and 2) cells compute the difference between their growth rate and the average
27 growth rate in the tissue. The model explains how growth inhomogeneity is smoothened over time through
28 a mechanical feedback (Fig.2B). A faster growing cell population is compressed and stretches the
29 surrounding slower growing cells consistent with experimental observations [52,55]. Since then the
30 mechanical feedback hypothesis has received much attention [56–60].

31 The mechanical feedback principle was subsequently coupled to the existence of a chemical morphogen
32 gradient to explain the apparent homogeneity of tissue growth and final growth arrest [59] (Fig.2C). Cells

beyond the reach of the Dpp gradient at the edge of the tissue grow less and lead to compression of cells inside. Consequently, cells at the periphery are stretched and divide, thereby expanding the tissue. Thus, mechanical compression of central cells would counteract the increased proliferation due to high Dpp and peripheral stretch would increase the proliferation capacity despite low Dpp levels, equalizing the growth rates across the tissue.

This general concept has been further elaborated in a 'Mechanical Feedback Model' that accounts for how mechanical stress leads to cessation of growth in a tissue where growth is induced by a morphogen gradient such as Dpp [58,60] (Fig.2C). Given that peripheral stretch persists and hence is not fully compensated by stretch induced growth in the periphery, central cells will become increasingly compressed by the peripheral region with increasing disc size. Hence, growth ceases once growth factors (like e.g. Dpp) cannot overcome this central growth inhibition due to tissue compression [58–60]. Control of disc size by mechanical feedback is yet to be evaluated experimentally.

The mechanical feedback provides an elegant mechanism to ensure uniformity of proliferation in the disc tissue despite the non-uniformity of morphogen distribution. Recent results support the role of mechanical feedback in ensuring a uniform proliferation pattern. Blocking the negative feedback loop via Hippo signaling caused heterogeneity of tissue growth [55].

The role of mechanical feedback on organ size control has been challenged by experiments in which central compression was increased by genetic modification of the basal membrane. Despite this increased compression the obtained fly wings were of normal size [61]. However, a subsequent study clearly indicated a role of cell density and compression on the regulation of cell proliferation via the mechanosensitive Hippo pathway [62]. Therefore, the mechanical feedback hypothesis has obtained support in ensuring uniform proliferation, but a role of the mechanical feedback hypothesis in size control requires further experimental testing. It remains to be shown that a modification of the cell area/pressure gradient indeed results in modified wing size. More importantly, it remains to be demonstrated that indeed compression or stretch results in reduced or elevated growth rates *in vivo*, respectively. Future approaches exploiting live-imaging will allow to obtain dynamic information on the growth dynamics of the disc and will shed light on the impact of tissue mechanics on tissue growth.

Metabolic constraint on growth

Both biochemical and mechanical control of growth operate at the tissue scale and eventually feedback onto cell growth and proliferation. Cell growth requires metabolic pathway to provide energy to fuel synthesis of mRNAs, proteins and lipids. The energy supply is provided by nutrients (carbohydrates,

1 amino-acids, etc.) and oxygen and are distributed in the body via specialized transport networks, the
2 vascular system in vertebrates and the tracheal system for oxygen in insects. As a consequence,
3 insufficient supply of oxygen and/or nutrients results in reduced body size [63,64]. In vertebrates and
4 invertebrates, energy storage organs (e.g. liver and fat body) function as relay and central sensors of
5 energy input in the diet. They elicit signals that may arrest growth on low diet. In flies, the fat body
6 triggers hormonal feedback at the heart of a systemic control that arrests growth at the end of development
7 when organs reach their approximate final size. Growing organs send signals that induce the hormonal
8 feedback growth arrest, and reduce fluctuating asymmetry. These mechanisms have been reviewed
9 extensively [1,65].

10 We consider here the possibility that oxygen and nutrient supply might become limiting factors of
11 developmental growth, and thereby affect tissue size independent of the hormonal feedback circuit. This is
12 based on observations that the total metabolic power or rate (in J/s or Watt) of an organism does not scale
13 linearly but sub linearly with body mass[66,67]. The relationship between metabolic rate (B) and mass (m)
14 shows allometric scaling. Max Kleiber measured the metabolic rate of mammals from the mouse to
15 elephants and found a power law across 6 orders of magnitude with exponent $3/4$ [68] (see Box 1).
16 Kleiber's law has been extended to many other organisms, from insects [69] to other metazoans [70–72].
17 The actual value of the exponent remains somewhat debated in invertebrates and smaller organism [73–
18 75] and some studies support rather a $2/3$ exponent [76]. Irrespective of the actual value of the exponent,
19 there is consensus that organismal metabolic rate scales sub-linearly with organism mass. As a
20 consequence, this implies that cells in larger organisms have a lower cellular metabolic rate than cells in
21 smaller animals. This observation is expected to impact the growth potential of an organism. Should the
22 Kleiber's law extend to the developmental phase of organism growth this would also affect developmental
23 growth. The metabolic power available to a cell sustains first cellular function (cell homeostasis) and any
24 surplus can be allocated for growth and the formation of new cells. The rate of energy demand for growth
25 scales linearly with cell number and organism mass. The sublinear scaling of metabolic power with
26 respect to mass has been proposed to reflect constraints imposed by the energy delivery through the
27 vascular system in particular the properties of a network that is space filling, minimizes dissipation and
28 with invariant termini due to the invariance of cell size and diffusion properties of oxygen [77,78]. As a
29 result, Kleiber's law implies a mismatch between energy demand and delivery and predicts a universal
30 bounded growth law (Box 1, C and D). It is tempting to speculate that such a mismatch could operate and
31 participate in the reduction of growth rate during development and size control.

32 Given the context of developmental tissue growth, Kleiber's law compares different organisms with
33 different size. It was shown to extend to other species such as *Planaria* [70]. Though the mass of an

organism is the sum of the mass of its organs, it is not clear whether individual organs follow the Kleiber law [79].

A study by West and colleagues investigated the relationship between cellular metabolic rates and whole organism size and metabolic rate [71]. They found that in highly dividing cells such as e.g. fibroblasts and epithelial cells the cellular metabolic rate is body size dependent. In contrast, in slowly dividing cells the metabolic rate is invariant with body size. This suggests that the supply limitations described by the West Brown and Enquist model of Kleiber's law maybe more restricted to growing and metabolically active tissues. Future studies will provide a comprehensive understanding of the relation between metabolic power and body mass especially investigating different stages of animal development and test if individual organs and tissues follow the Kleiber law.

Conclusions and perspectives

The growth of organs and tissues during development is controlled by local (e.g. organ) size sensing mechanisms. Following chiefly the literature from *Drosophila*, we considered two major information modules that participate in organ size sensing namely biochemical patterning and mechanics (Fig.3). The disc-specific biochemical and mechanical modules function through local feedback mechanisms whereby a proxy for tissue size affects cellular growth and division. It is striking that while evidence support the existence of feedbacks, none of them appears sufficient to control organ size. The hormonal systemic feedback circuit that gates developmental progression and growth arrest at the end of *Drosophila* larval development provides yet another layer of control [4,80]. In *Drosophila*, chemical and mechanical organ size sensing mechanisms are thought to induce systemic hormonal feedback control that arrest growth but the nature of the signals remain elusive [1] though, interestingly, Dpp has been suggested to be involved [81]. The picture that emerges from the literature is that proper size regulation requires different layers of regulation namely genetic patterning, mechanics and hormonal signaling.

In addition, we discussed the potential role of energetics in limiting growth during development due to competition and mismatch between energy supply through the vascular transport system, and energy demand which predict bounded growth. Assessing this possibility will require microcalorimetry studies during development as pioneered in zebrafish [82] and *Planaria* [70].

It will be important to test whether energy supply through the transport system is limiting and participates in growth arrest along with mechanics and patterning. Interestingly the general architecture of tubular networks like the tracheal system in insects or the mammalian vascular system is governed by feedback-mechanisms that fine tune network structure in areas deprived of oxygen or nutrients [83,84]. We are only

beginning to explore the potential role of feedback-regulation of energy supply and metabolism during developmental growth.

Individual cells are the basic unit of growing tissues and hence, future investigations will need to focus more on the cellular scale *in vivo*. Moreover, by definition growth refers to volume and mass increase over time. *In vivo* studies have so far used indirect proxy for growth such as cell division, or the apical cross section area of cells, or clones of cells. It is essential to develop methods to extract volume measurements at the cellular scale. Equally important is the need to measure time evolution of these parameters through the cell cycle during the whole process of organ growth. It will become possible to correlate cell and tissue growth with calorimetric studies that assay the energetic of tissue growth. With this new phenomenology it will become possible to assess in new ways how patterning, mechanics and energetics are integrated with systemic, hormonal feedbacks to control growth and growth arrest, and to disentangle the feedback mechanisms that underlie tissue and organ growth during development (Fig. 3).

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Competing interests

The authors declare no competing financial interests.

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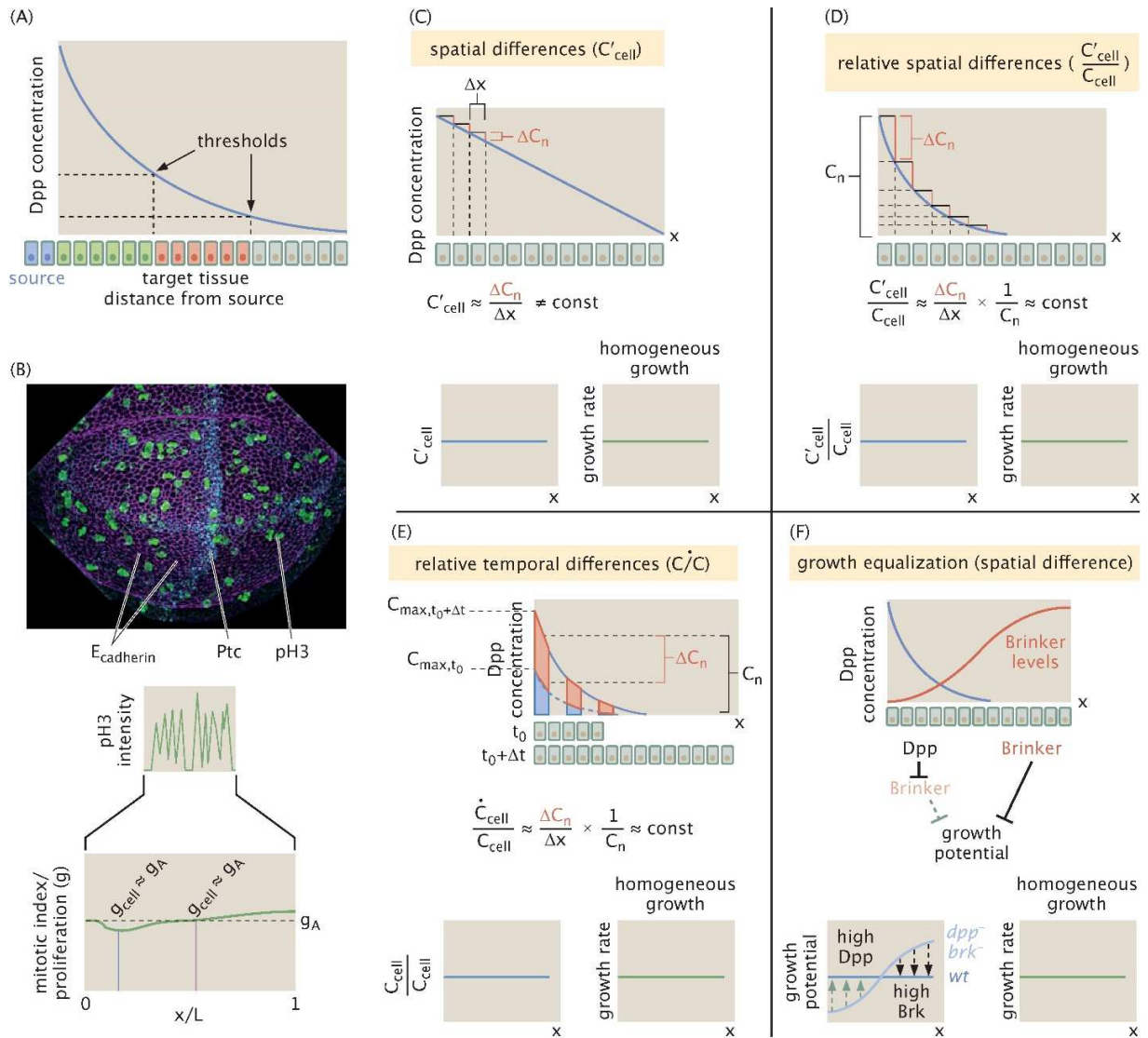


Figure 1 – Growth control by biochemical signaling gradients

(A) Morphogens from concentration gradients from their source. They induce different target genes (e.g. green, red and grey) in a concentration and hence distance-dependent manner. (B) A *Drosophila* wing disc labeled for phospho-Histone-H3, a marker for cells in mitosis. The proliferation rate is roughly uniform in the tissue (bottom). It remains debated how graded morphogen input can result in uniform proliferation output. (C) According to the linear slope model each cell n evaluates the spatial differences in Dpp levels (C'_{cell}). Assuming a linear slope of the gradient, spatial differences are uniform in the tissue (bottom left) leading to homogeneous growth (bottom right). (D) Given the exponential shape of the Dpp gradient, in the Exponential Decay Slope Model cells compute the relative spatial differences in Dpp levels (C'_{cell} / C_{cell}) which is spatially uniform. (E) The Temporal Dynamics Model is based on the observation that Dpp production increases in time ($t_0 + \Delta t$) and that the gradient scales in space and time. Therefore, the relative

1 temporal increase in Dpp levels is uniform (bottom, left) leading to uniform growth (bottom, right). (F)
2 Dpp signaling represses transcription of Brinker in the central region thereby leveling out a non-uniform
3 growth potential along the disc tissue: In *dpp⁻ brk⁻* double mutants, growth is increased in the periphery
4 and decreased in the center (bottom left, light blue). In contrast, in the wild type (*wt*) the central growth
5 potential is elevated due to absence of Brinker and presence of Dpp while the peripheral growth potential
6 is reduced by presence of Brinker (bottom left). This yields a uniform growth rate along the disc tissue
7 (bottom right).

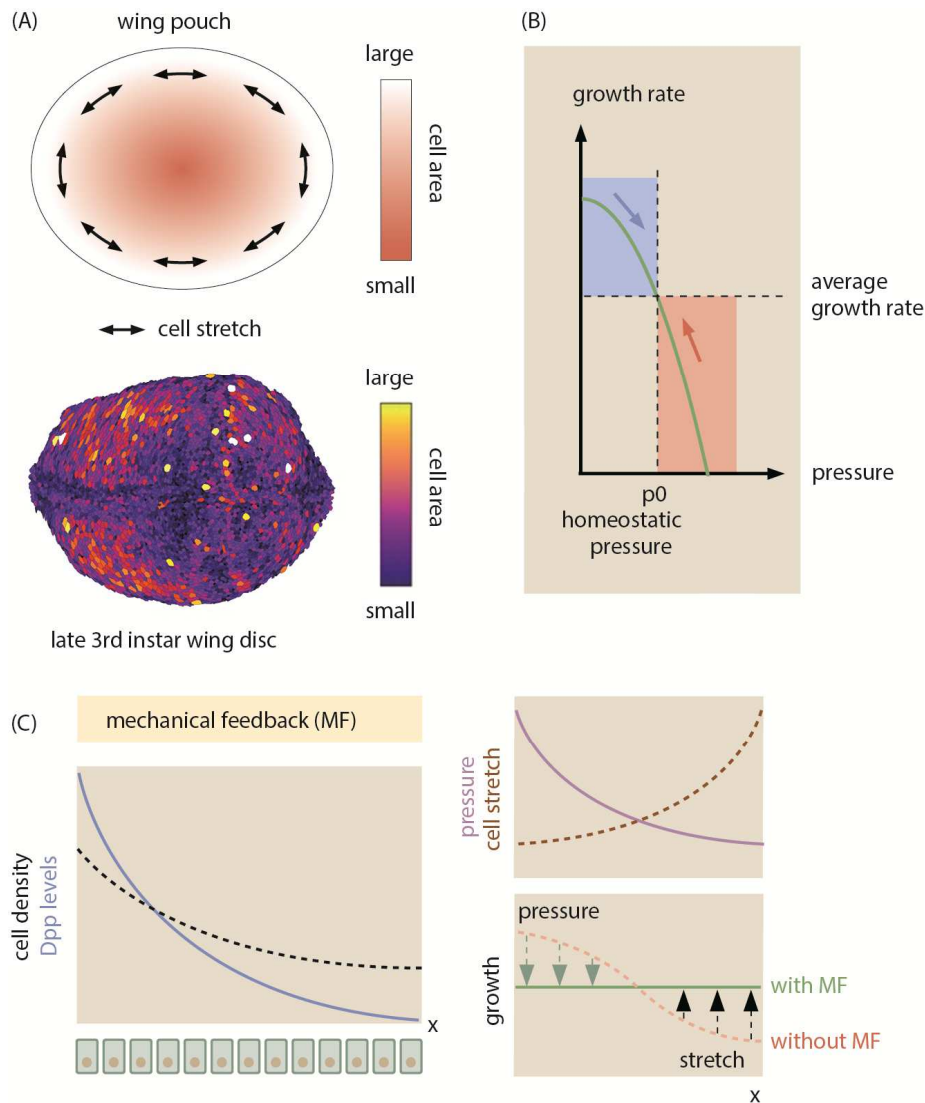


Fig.2 – Growth control via mechanical-feedback (MF)

(A) **top:** During wing disc growth cell area becomes graded with small cells in the center and large cells in the periphery. In addition, peripheral cells tend to be stretched in tangential orientation. **bottom:** Cell outlines of a fully grown wing imaginal disc color-coded for cell area. (B) The effect of mechanical feedback on cellular growth: The green line indicates the relationship of the cellular growth rate and cellular compression. Increasing pressure will result in a quick drop of the cellular growth rate. Cells are driven towards a homeostatic pressure p_0 . The pressure will evolve based on the difference between the local growth rate and the average growth rate of cells in the neighborhood. If the cell growth rate is larger than the average, the pressure increases until p_0 (blue) and if the growth rate is lower than the average, the pressure will decrease until p_0 (red). (C) The mechanical feedback model is based on the findings shown in panels A and B. Higher central cell density and hence pressure decreases the growth and proliferative

1 advantage provided to central cells by high Dpp levels. In contrast, stretch in peripheral cells increases the
2 proliferative capacity to compensate for lower Dpp input. In combination, both mechanical feedbacks
3 (stretch and compression) equalize the growth profile (bottom, right).

4

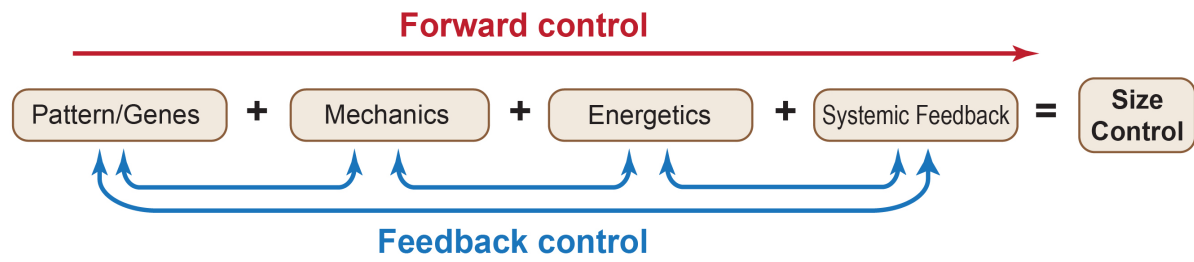
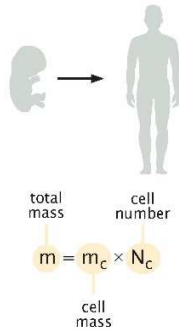


Fig.3 – Layers of growth control linked by inherent feedback loops

Size control is the outcome of combinatorial input from a multi-layer control system consisting of patterning input, mechanics and energetics and systemic feedback from the nutrient sensing system. While individual layers provide hard wired, genetically encoded Forward Control to ensure proper size, interconnectivity via Feedback Control allows crosstalk and integration of information from all layers. The collective input from all layers provides robust and reproducible sizing information during tissue growth control.

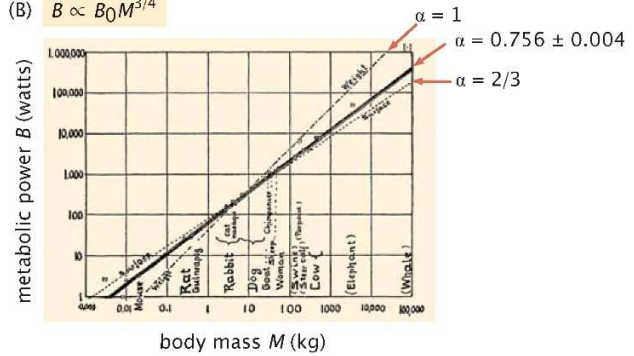
(A) METABOLIC POWER



$$B = N_c B_c + E_c \frac{dN_c}{dt}$$

cell metabolic rate
energy required to produce a new cell
power allocated to sustain organism
power allocated to growth

(B) $B \propto B_0 M^{3/4}$



(C)

GROWTH EQUATION

$$\frac{dm}{dt} = \left(\frac{m_c}{E_c} \right) B - \left(\frac{B_c}{E_c} \right) m$$

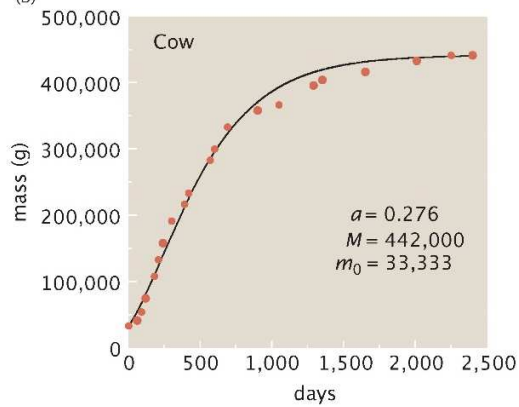
KLEIBER LAW

$$B = B_0 m^{3/4}$$

$$\frac{dm}{dt} = \underbrace{am^{3/4}}_{\propto \text{energy supply}} - \underbrace{bm}_{\propto \text{energy demand}}$$

→

(D)



Box 1 – Limitations imposed by the Kleiber's law on organism growth

(A) Organism mass m is the product of cell number N_c and the average mass of a single cell m_c . The metabolic rate B of an organism consists of the power allocated to cell maintenance (homeostasis) ($B_c N_c$, where B_c is the cell metabolic rate) and the power allocated to cell growth, which depends on the cost of producing a new cell E_c and the number of newly formed cells dN_c per time interval dt . (B) Kleiber's law states that B scales sub-linearly with the asymptotic body mass M (final animal mass) and this relation can be described by a power law with exponent $\alpha=3/4$. B_0 represents a constant for a given animal species. Kleiber originally plotted asymptotic mass versus metabolic power (log scale) for a wide range of different animals, ranging from mice to whales. (C) The growth equation describes the rate of organism mass increase and follows directly from the expression of organism metabolic rate B and organism mass m in (A). Considering Kleiber's law for the sub-linear scaling of B with body mass, the growth equation shows a mismatch between energy demand (bm), which is proportional to body mass ($b \equiv B_c/E_c$), and energy supply ($am^{3/4}$) which scales sub-linearly with body mass ($a \equiv B_0 m_c/E_c$) due to constraints associated with the vascular system [77]. Therefore, the energy demand increases faster than the supply during animal growth. This predicts a steady state with asymptotic mass $M = (a/b)^4$ (D) This demand-supply discrepancy might be responsible for the sigmoidal shape of animal growth curves, as shown here for the cow. While initially body mass increases rapidly, mass increase quickly decelerates and

1 approaches the asymptotic animal mass. This analysis concerns ontogenic growth based on Kleiber's law.
2 The hypothetical impact of this on developmental growth rests on the validity of sub-linear scaling of
3 metabolic power to embryo mass during development (or its extension to internal organs), and the
4 assumption that cell mass is invariant. In *Planaria*, Kleiber's law is valid but the cell mass is a function of
5 organism size [70].
6