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REVIEW

Mechanical control of growth: ideas, facts and challenges

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ABSTRACT

In his classic book *On Growth and Form*, D'Arcy Thompson discussed the necessity of a physical and mathematical approach to understanding the relationship between growth and form. The past century has seen extraordinary advances in our understanding of biological components and processes contributing to organismal morphogenesis, but the mathematical and physical principles involved have not received comparable attention. The most obvious entry of physics into morphogenesis is via tissue mechanics. In this Review, we discuss the fundamental role of mechanical interactions between cells induced by growth in shaping a tissue. Non-uniform growth can lead to accumulation of mechanical stress, which in the context of two-dimensional sheets of tissue can specify the shape it assumes in three dimensions. A special class of growth patterns – conformal growth – does not lead to the accumulation of stress and can generate a rich variety of planar tissue shapes. Conversely, mechanical stress can provide a regulatory feedback signal into the growth control circuit. Both theory and experiment support a key role for mechanical interactions in shaping tissues and, via mechanical feedback, controlling epithelial growth.

KEY WORDS: Stress, Growth, Mechanics, Hippo

Introduction

In *On Growth and Form*, first published 100 years ago, D'Arcy Thompson famously considered the study of growth and form of plants and animals to be in the domain of physics and mathematics, stating: 'the morphologist is, *ipso facto*, a student of physical science' (p. 8, Thompson, 1917). Yet, our present understanding of animal and plant development owes much more to genetics, cell biology and biochemistry than to physics and mathematics. The immense progress of modern developmental biology has provided us with a great deal of insight into the genetic and molecular basis of developmental processes and the biochemical signals that control them. However, a century later, the central question of how controlled growth defines biological form, sublimated from D'Arcy Thompson's writings, remains unanswered. Hence, it seems timely to revisit D'Arcy Thompson's agenda of understanding physical and geometric aspects of morphogenesis in the context of our current knowledge of the cellular and molecular genetic aspects of developmental biology.

Starting on this path, one immediately arrives at the problem of mechanics in development, which has attracted considerable recent attention (Coen et al., 2004; Heer and Martin, 2017; Heisenberg and Bellaïche, 2013; Lecuit and Yap, 2015; Munjal and Lecuit, 2014; Tallinen et al., 2016). In this Review, we begin with an almost literal interpretation of Thompson's dictum '…the form of an object is a "diagram of forces"', to describe a specific mathematical framework relating geometric form to the history of the growth process. This relation will immediately require assumptions concerning tissue mechanics and will illustrate that some spatiotemporal patterns of growth might be in certain ways mechanically 'better' than others. This will in turn bring us to the question of controlling growth as a function of spatial position and time in order to achieve correct developmental outcome: a correctly shaped and sized organ or limb. We discuss this in the context of a biologically well-studied model of organ growth, the *Drosophila* wing, and with particular reference to a signaling network – the Hippo pathway – that integrates multiple molecular signals with mechanical input. We also discuss the issues that one encounters in connecting cellular scale cytoskeletal mechanics, which provides a mechanosensor linked into growth control, with the mechanical behavior on the scale of the tissue. Our Review is not intended as a general overview of growth control mechanisms, but rather an argument for, and an exposition of, a fundamental role for tissue mechanics in growth control.

A mathematical connection between 'growth' and 'form'

If we consider a change in form that accompanies growth, such as a ball of cells in a limb bud growing into an elongated appendage, we can anticipate that there may be many different spatiotemporal patterns of growth that arrive at the same geometric shape. Can we begin to classify different growth strategies? How can these strategies be plausibly 'encoded' in cell behavior during the developmental process to yield a reproducible shape? For simplicity, we proceed by considering an abstract two-dimensional (2D) problem, which will illustrate the main ideas that can be applied in specific biological contexts, and which generalizes naturally to 3D.

Consider a growing 2D planar 'body' defined by the shape of its boundary. Suppose the initial body has a shape of a disc: what growth process could create a given final shape? Two types of growth processes can immediately be imagined: (1) 'boundary growth' corresponding to proliferation of cells only at the edge of the body, and (2) 'bulk growth' that occurs throughout the body. In either case, to understand the overall shape of the body we have to understand the motion of the boundary, induced by growth. Both types of growth exist in nature, with boundary growth typically observed for hard tissues such as bone, antlers and shells, and bulk growth typically observed for soft tissues such as heart or intestine (Cowin, 2004). Here, we focus our discussion on the latter, bulk growth scenario, because it is commonly observed and it illustrates the role of mechanics in relating the process of growth to the resulting shape. In the mathematical discussion that follows, terms used in the equations are defined in Box 1, and the supplementary information provides further information to the interested reader.

In the simplest case, growth is locally isotropic – which is to say cell division axes are randomly distributed – and can be described simply by the rate of cell division $\gamma(\vec{r}, t)$, which can vary depending upon position, $\vec{r}$, within the body and time, t. As cells grow and divide, the total volume (or area in our 2D case) of the body will

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Box 1. Glossary of mathematical symbols

γ	growth rate
r	position
t	time
ρ	label identifying a particular cell
V	velocity vector
u	displacement vector
s	strain tensor
σ	stress tensor
μ	elastic modulus
δ	Kroneker delta
τ_σ^{-1}	stress relaxation rate
m_{ab}	myosin activity
M	growth factor concentration

Box 2. Glossary of physical and mathematical terms

Cellular flow. Change in position of a cell over time.

Conformal map. A transformation of a planar object that preserves local angles.

Einstein's convention. A mathematical notation that abbreviates formulas by implicit summation of repeated indices.

Elastic. An object or material that resists being compressed or stretched and returns to its normal shape after external force is lifted.

Elastic modulus. The ratio of stress to strain, which describes how deformable a material is in response to an applied force.

Harmonic function. A function for which second order partial derivatives sum to zero (i.e. satisfy the Laplace equation).

Harmonic/stressless growth. Growth in a harmonic spatial pattern, which generates no change in stress.

Strain. Local deformation of a material arising from spatially varying displacement of its elements.

Strain tensor. A mathematical description of local deformation based on the spatial variation of vectorial displacements of material points. In two dimensions, spatial components of a tensor form a 2×2 matrix.

Stress. Force per unit area that components of a material exert on each other.

Stress tensor. A mathematical description of a force vector acting on the small element of the surface with a given spatial orientation.

Velocity vector field. An area (in 2D) or volume (in 3D) in which each point is assigned a vector indicating its speed and direction.

increase, but what will be the shape? The answer depends on the pattern of growth, but also on the details of tissue mechanics. In particular, it matters how deformable cells are, how much they stick to each other, and how free they are to rearrange and 'slide' by each other. Independent of the details, however, growth results in a slow displacement of cells that can be described by a velocity vector field (see Glossary, Box 2) $\vec{V}(\vec{r},t) = [V_x(\vec{r},t),\ V_y(\vec{r},t)]$ as a function of position within the body (we will often use an alternative notation $V_a(\vec{r},t)$ using index a as a stand-in for either x or y components of a vector). Given this cell displacement velocity field as a function of time, one can determine the positions of all cells as they are carried along within the mass of proliferating cells. The positions of cells in the body $\vec{r}(\rho,t)$ are governed by:

$$\frac{d}{dt} r_a(\rho,t) = V_a(\vec{r}(\rho,t),t), \qquad (1)$$

where ρ identifies a particular cell within the body and the change in its position over time is described by the vector field $V_a(\vec{r},t)$, which we shall refer to as cellular flow (see Glossary, Box 2) (Fig. 1A). To understand how growth impacts form we need to know: given a prescribed local rate of growth $\gamma(\vec{r},t)$ per unit area, what is the resulting cellular flow $\vec{V}(\vec{r},t)$? The answer depends on our assumptions about tissue mechanics.

Let us begin by considering the limit of purely elastic tissue (see Glossary, Box 2), in which cells are not free to rearrange, but instead deform, generating elastic forces. Biologically, this could correspond to an epithelial tissue in which individual cells resist deformation, while tightly adhering to each other, and do not rearrange or delaminate. In this case, tissue mechanics at any moment of time is effectively that of an elastic rubber sheet, but a peculiar one, in which material can be added internally thanks to the growth and proliferation of cells. It is intuitively plausible that a uniformly growing tissue will gradually increase in size without changing shape. A uniform 'inflation' due to uniform bulk growth will not generate any mechanical stress (see Glossary, Box 2) within the body. On the other hand, an arbitrary non-uniform growth would be expected to generate stress, as the regions that have locally expanded more than their surroundings push on their neighbors, compressing them in the direction of expansion, while stretching them along the perpendicular axis to accommodate the increased area of the overgrowing region (Fig. 2A).

Theory of elasticity tells us that mechanical stress is proportional to local deformation (strain; see Glossary, Box 2) (Fig. 2B), and provides a mathematical description for it in the form of local strain and stress tensors (see Glossary, Box 2), which keep track of the types of local deformation and of the direction of resulting forces. The strain is described mathematically by a tensor $s_{ab}(\vec{r}) = \partial_a u_b(\vec{r}) + \partial_b u_a(\vec{r})$, where indices a and b stand in for (x or y) vector and tensor component labels, so that vector $u_a(\vec{r})$ denotes the displacement of a material point (of the elastic sheet) away from its unperturbed position and ∂_a denotes a partial derivative with respect to r_a. Thus, different components of s_{ab} are defined by how components of the displacement vector u_a change with position (Fig. 2C). In the simplest case, the elastic stress tensor, which defines the forces with which adjacent elements of an elastic sheet act on each other, is simply proportional to the local strain, $\sigma_{ab}=\mu s_{ab}$, where μ is the elastic modulus (see Glossary, Box 2), which describes how deformable the material is. (For simplicity, we assumed here that shear and bulk moduli are equal to each other and are given by μ. Relaxing this assumption does not change the conclusions of our analysis.)

Strain in a growing elastic tissue arises from the displacement of cells relative to their neighbors, due to non-uniformity of cellular flow. At any given instant the change in cell displacement is given by flow velocity, $du_a/dt=V_a$, so spatial derivatives of the cellular flow field define the rate of strain $ds_{ab}/dt=\partial_a V_b+\partial_b V_a$. We expect temporal changes in strain to generate proportional changes in stress. However, if the elastic sheet – live tissue in our case – is growing from within, the proliferation of cells can partially offset the straining (e.g. stretching can be relieved by local cell proliferation, which increases the number of cells within the expanded area) resulting in the following equation for the temporal rate of change of local stress tensor:

$$\frac{d}{dt}\sigma_{ab} = \mu[\partial_a V_b + \partial_b V_a - \delta_{ab}\gamma], \qquad (2)$$

where the first two terms on the right-hand side define the rate of strain, and the last term is the offsetting contribution of an isotropic growth rate. The symbol δ_{ab} is the 'Kroneker delta', which is equal to 1 for $a=b$ and zero otherwise. Its appearance here is the expression of the assumed isotropy of growth. In the supplementary information we give more general forms of Eqn 2, relaxing a number of simplifying assumptions that we have made

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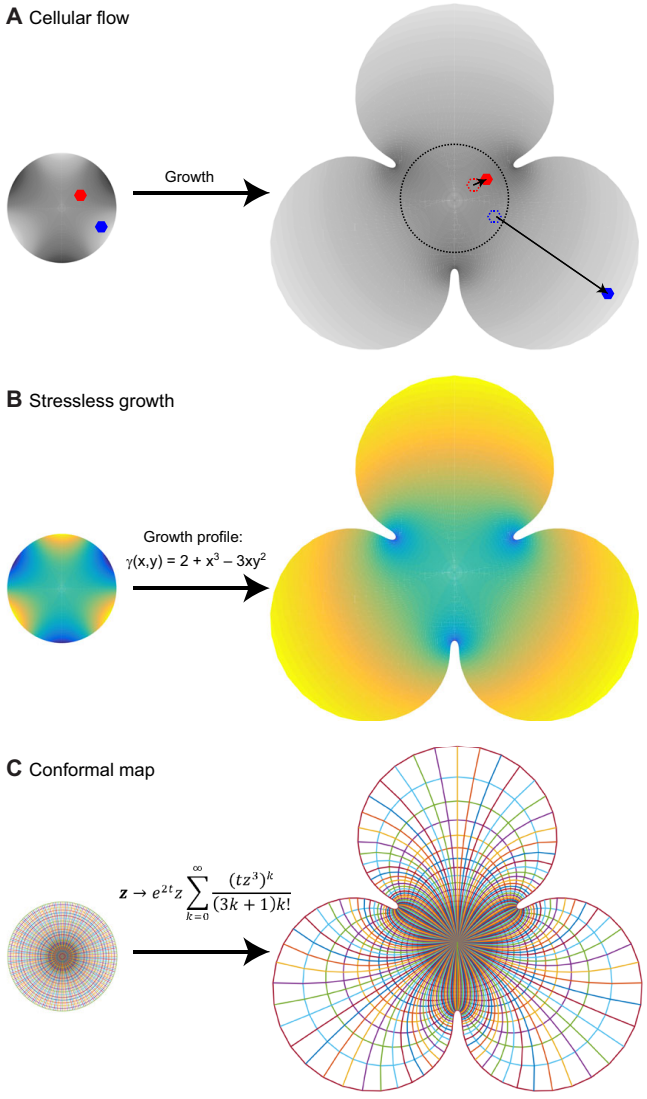

Fig. 1. A toy example of conformal growth. (A) Individual cells (illustrated by blue or red hexagons) move as the tissue changes shape as a result of growth: a circular body (gray) (left) undergoing an imprinted growth profile (shown by the gray shading) leads to a more complex shape (right). Dashed outlines indicate the initial locations of the body and cells. 'Cellular flow' corresponds to the continuous displacement of cells as a function of time. (B) A harmonic growth pattern, $\gamma(x, y)=2+x^3-3xy^2$, which is the simplest 3-fold symmetric harmonic function, was 'imprinted' in the circular body (left), defining the alternating sectors of faster (yellow) and slower (blue) growth. It was assumed that growth rate remains constant along the trajectory of each point. Resulting growth greatly expands the domain of faster growth compared with the slow-growing regions, changing the shape of the 2D body (right). (C) The conformal mapping of initially polar coordinates onto the final shape. The conformal map is given by $F(z,t) = e^{2t}z \sum_{k=0}^{\infty} \frac{(tz^3)^k}{(3k+1)k!}$, where $z=x+iy$ is a complex number constructed from spatial coordinates (x, y) generated by the growth profile γ specified above (see supplementary information part D). To relate this continuum analysis with growth of cellular tissue, one would assume that growth rate is constant along cell lineage, with newborn cells growing at the rate that interpolates the growth rate of their neighbors.

so far: e.g. we allow for non-equal elastic moduli (supplementary information part A) or allow for anisotropic tissue growth (where cell division has preferred orientation) and for plastic relaxation of stress (supplementary information part B), which could occur due to cell rearrangement and cell shape changes. These generalizations allow for a more realistic description of tissue mechanics. Nonetheless, the simple, 'minimal', model introduced above, suffices to introduce key ideas, which can then be readily extended to more realistic and elaborate models. Moreover, we emphasize that at least in some well-studied animal tissues, it is clear that stress relaxation mechanisms are insufficient to prevent accumulation of stress induced by genetically altered growth rates (Legoff et al., 2013; Mao et al., 2013; Pan et al., 2016). This indicates that accumulation of stress in response to growth is not just a theoretical possibility, but a biological reality.

Eqn 2 governs the dynamics of stress, but to determine $\vec{V}$ one needs an additional assumption, namely that cellular flow generated by growth proceeds in approximate mechanical force balance. This assumption is appropriate because the growth process is slow compared with the time scales of tissue mechanics. Laser ablation of cell junctions has revealed that epithelial cells in tissues such as a *Drosophila* wing imaginal disc are under tension. Cells move within a fraction of a second after cutting, suggesting that local response to the loss of mechanical equilibrium is at least two orders of magnitude faster than movements observed during growth (Farhadifar et al., 2007). Mechanical balance means that net local force in the bulk must be equal to zero at all times. As net force per unit area at any point of a sheet is given by the divergence of the stress tensor ($\partial_b\sigma_{ab}$), one then has:

$$\partial_b\sigma_{ab}(\vec{r},t) = 0 \qquad (3)$$

[following Einstein's convention (see Glossary, Box 2), repeated indices are being summed over]. Combining Eqn 2 and Eqn 3 one can derive the desired equation relating cellular flow to the growth profile:

$$\nabla^2 V_a + \partial_a\partial_b V_b = \partial_a\gamma, \qquad (4)$$

where $\nabla^2 = \partial_x^2 + \partial_y^2$ and $\partial_b V_b$ is the divergence of $\vec{V}$. Solving Eqn 4 to determine cellular flow, $\vec{V}(\vec{r},t)$, in terms of growth rates, $\gamma(\vec{r},t)$, and integrating the flow over time will relate the shape of the (2D) body to the history of growth. Thus, the simplifying and plausible assumption of force balance enables one to relate 'growth' to 'form'.

3D shapes formed by 2D growth

Most non-uniform patterns of growth will introduce stresses that accumulate as our planar 2D body grows. One mechanism to relieve the accumulation of in-plane stress in an elastic sheet is through buckling of the sheet out of plane. This will occur if the in-plane stress is sufficiently high to overcome the energetic cost of bending, which increases with the thickness of the shell. Bending or folding of epithelial sheets is a common morphogenetic process during animal development, and differential growth could be one way to generate forces that promote this.

The study of 3D shapes formed by buckling of thin 2D sheets has been an area of exciting recent progress in physics, combining theory of non-Euclidean shells with new experimental demonstrations (Audoly and Boudaoud, 2003; Dervaux and Ben Amar, 2008; Efrati et al., 2013; Lewicka et al., 2014; Marder et al., 2007; Santangelo, 2009; Sharon and Efrati, 2010). A non-Euclidean shell is a thin elastic shell for which the intrinsic 2D geometry is incompatible with a flat configuration. This incompatibility results in internal stress in a flat configuration, unless the shell is allowed to

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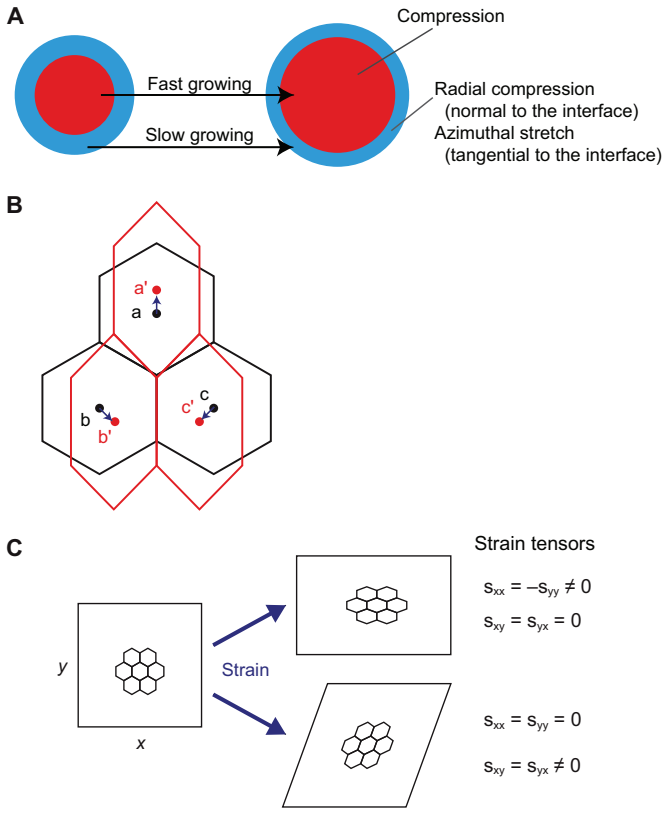

Fig. 2. The effects of displacement and strain. (A) Faster growth of the inner (red) region of an elastic tissue layer results in compression of the faster-growing region and causes radial compression and azimuthal stretching of the surrounding (blue) region. (B) A cellular perspective on strain. Deformation of cells from the initial shape (black) to a later shape (red) is associated displacement of their centroids a,b,c. (C) Continuum elasticity describes tissue deformation on supracellular scale. These deformations can be described mathematically by strain tensors. To illustrate this, we show two modes of deformations that do not change the area of an arbitrary region, and how this is related to components of the strain tensor (s).

deform into a 3D shape. A more quantitative description is based on the celebrated *theorema egregium* of Gauss that relates intrinsic metric (local distance measure) of the surface to its extrinsic Gaussian curvature (Audoly and Pomeau, 2010). The effect of non-uniform growth within an elastic sheet is mathematically equivalent to a change in intrinsic metric and hence results in a change in local curvature of the relaxed 3D shell (see supplementary information part C). Local Gaussian curvature, which is the product of the two local radii of curvature, does not fully constrain the embedding of the surface in 3D (e.g. a sheet of paper gently bent into a cylinder still has its Gaussian curvature equal to zero), so the actual shape of the surface is defined by additional minimization of the bending elastic energy (Audoly and Pomeau, 2010; Sharon and Efrati, 2010). The mechanism of shaping 3D surfaces by non-uniform local expansion was strikingly illustrated by recent experiments with non-uniformly swelling patterned hydrogels (Gladman et al., 2016; Na et al., 2016).

Of course, the role of bending and the finite thickness of tissues open up other important mechanisms for biological control: differential growth on the two sides of a multilayered sheet, and the apical constriction of the polarized epithelial layer, which play key roles in defining 3D shapes during morphogenesis (Polyakov et al., 2014; Savin et al., 2011; Sweeton et al., 1991).

Planar 'harmonic growth'

Remarkably, an elastic tissue sheet can also grow in the bulk without generating any stress whatsoever. This possibility may seem counter-intuitive, but there is a strikingly simple way: it just requires a certain constraint on the growth profile $\gamma(\vec{r},t)$; specifically, as derived below, the instantaneous growth profile has to be a harmonic function of position (see Glossary, Box 2), which is to say that it must satisfy the Laplace equation, $\nabla^2\gamma(\vec{r},t)=0$. The latter condition is trivially satisfied by uniform growth, which produces a simple 'dilation' of the tissue: an increase in size without a change in shape. Yet, there is a large class of non-trivial solutions as well, which provide a direct connection between the spatiotemporal pattern of growth and the 2D shape.

Suppose the rate of displacement $\vec{V}(\vec{r},t)$ of cells is such that it accommodates cell proliferation and growth without changing cell density:

$$\partial_x V_x + \partial_y V_y = \gamma(\vec{r},t), \tag{5}$$

which expresses the fact that the flow of cells out of any small square region is exactly equal to the rate of cell birth in that region. Suppose that, in addition, the flow has some special properties:

$$\partial_x V_x = \partial_y V_y \text{ and } \partial_x V_y = -\partial_y V_x. \tag{6}$$

It follows from Eqn 6 that $\nabla^2 V_a = 0$ and one readily sees (by substituting Eqn 5 into Eqn 4) that Eqns 5 and 6 suffice to ensure that mechanical balance condition Eqn 4 is satisfied. Furthermore, one observes that $\partial_x V_x = \partial_y V_y = \frac{1}{2}\gamma(\vec{r},t)$ and $\partial_x V_y + \partial_y V_x = 0$, which also follow from Eqn 5 and Eqn 6, make the right-hand side of Eqn 2 vanish, so that any growth-driven displacement of cells, described by the velocity field $\vec{V}(\vec{r},t)$ that satisfies Eqns 5 and 6, generates no elastic stress. Self-consistency of Eqns 5 and 6 requires $\gamma(\vec{r},t)$ itself to satisfy the constraint of solving $\nabla^2\gamma(\vec{r},t)=0$, the Laplace equation. Solutions of the Laplace equation are called harmonic functions and we shall refer to growth generated by harmonic patters of γ as 'harmonic growth' (see Glossary, Box 2). The simplest case of harmonic growth $\gamma(\vec{r},t)$ is a constant, but non-uniform growth profiles can also be harmonic. One such example is given in Fig. 1.

To understand better the connection between harmonic growth and shape, we can consider the map between the positions of the cells before and after a short period of growth. Cell displacement flow due to a harmonic pattern of growth generates a very particular map between initial and final positions of cells: a conformal map (see Glossary, Box 2). A conformal map of a plane deforms lines but preserves angles of their intersections so that any orthogonal grid remains orthogonal, as illustrated by Fig. 1C. Conformal maps were invoked by D'Arcy Thompson in his 'transformations' relating animal shapes (discussed by Abzhanov, 2017), and can be understood as outcomes of non-uniform local dilations of the plane (see supplementary information part D for a more precise exposition).

Now, it is a mathematical fact that any smooth 2D shape can be conformally mapped into any other smooth shape. It can also be shown that conformal maps are generated by growth satisfying the harmonic constraint (see supplementary information part D), providing a clear mathematical blueprint for a spatiotemporal pattern of growth that takes the (2D) tissue from some initial shape, to a final shape. Based on this blueprint, Fig. 1 provides an illustration of how a moderately complex shape can be generated by growth of a disc-shaped body with the position-dependent growth rate 'imprinted' on cells at the initial stage.

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We have arrived at the harmonicity constraint by requiring that growth does not generate any additional stress (i.e. $\frac{d}{dt}\sigma_{ab} = 0$) so the harmonic, or conformal, growth process is effectively 'stressless'. This, however, does not mean that the tissue itself harbors no stress, as it is possible that the growth process that generated the tissue was not always harmonic (or that the stress is induced by external forces). The former possibility is particularly interesting because residual stress could act as an effective regulator of growth and could guide and coordinate the growth process to achieve harmonicity such that no additional stresses are generated.

Throughout biology, one can find examples of flat sheets of cells that grow without evident accumulation of stress (as it would otherwise lead to buckling, or distortions of cell shape). Uniform growth has been well-described in some tissues, such as the *Drosophila* imaginal discs, but do other harmonic growth patterns occur in nature? Alim et al. (2016) used conformal mappings relating the shapes of a growing plant leaf at different times to estimate the spatial distribution of growth on the leaf surface, subsequently comparing it with the direct measurement of growth. The result for the two plants examined (*Petunia* and tobacco) was indeed close to conformal. Furthermore, deviations from conformality were clearly associated with local anisotropy of growth, which was particularly evident near leaf veins. Although anisotropy of growth violates conformality, it provides an additional biologically relevant handle for growth control (Coen et al., 2004).

The example of stressless, harmonic growth in 2D provides perhaps the simplest physically plausible and mathematically explicit connection between growth and form, showing how any form can be generated by an appropriate growth pattern, without an accumulation of stress. It also leads us to suggest that in most instances involving flat tissues (i.e. when buckling is not desired), harmonic growth profiles would be preferred in biological systems. However, complex shapes would require elaborate growth profiles and the question therefore becomes: how could these be specified and constrained to be harmonic functions at all times? Are there biologically plausible ways to generate such growth profiles?

Controlling growth profile with stress

Rates of growth and proliferation generally depend on cell identity, which is determined by both lineage and a variety of intercellular signals. Our example in Fig. 1 assumed that growth rate is constant along cell lineage and took advantage of the special property of conformal maps: conformal mapping preserves the harmonic property so that if the growth profile is harmonic at t=0, it will stay harmonic at all times. Yet, this lineage-dependence mechanism does not offer much possibility for additional control of growth rates and it is well known that biological growth is also controlled by external factors, such as local concentrations of growth factor 'morphogens' and other signals. These are not directly specified by lineage but rather depend on position and time via the growth factor concentration $M(\vec{r}, t)$. Although it is possible to contrive scenarios in which patterns of diffusible growth factors would themselves obey the harmonicity constraint, we argue that tissue mechanics presents a more robust way of coordinating local growth to mitigate its mechanical consequences and prevent undesired tissue deformations.

Mechanical feedback on growth

Generally, one might expect that growth factor signals will not conform to the constraint required for harmonic growth. A simple solution to the resulting mechanical 'conflict' between locally overgrowing or undergrowing patches of tissue could be provided by mechanical feedback arising from possible dependence of the rate of growth on mechanical stress σ in addition to its dependence on growth factors, i.e. $\gamma(M, \sigma)$ (Shraiman, 2005).

In the simple case of elastic tissue, we showed how growth in general leads to accumulation of stress (Eqn 2), except when γ obeys the harmonicity constraint. If growth rate γ monotonically decreases with increasing compression, excess local growth would generate excess compression, which could feed back as a signal to decrease local growth rate. Conversely, insufficient local growth could result in local tension, increasing the growth rate. Steady growth would be achieved when the stress distribution does not change, which happens when the growth profile becomes harmonic. Mechanical feedback was originally proposed as a mechanism for achieving uniform growth of tissues (Shraiman, 2005), but it could also work for other harmonic growth profiles.

Our consideration of the mechanical implications of growth thus leads us to suggest that a regulatory connection between mechanical stress and growth provides a simple and robust mechanism for ensuring harmonic, stressless growth. Recent studies of biological growth are consistent with this view, and have begun to give us insight into the molecular mechanisms by which stress and growth can be linked. Although the above argument was formulated in the context of oversimplified elastic description of tissue, we shall argue below that our considerations readily extend to a more complex and realistic view of epithelial tissue as an active and adaptive medium.

Mechanical feedback through active mechanics

Live epithelial tissues exist in a state of internal stress generated by contractility of the actomyosin cortex. External forces shift the cellular force balance and can cause an adaptive reorganization of the cellular cytoskeleton. Biologically, this reflects the fact that cells can respond to a change in stress in ways that resist that change. For example, cells that are stretched increase their F-actin and myosin levels in a manner that helps them counter the stretch they are experiencing (Kasza and Zallen, 2011). The resulting active response can be expected to deviate from simple elastic and even visco-elastic behavior.

To introduce active, myosin-driven, internal tension we generalize the description of stress dynamics (Eqn 2) to allow for the action of myosin and the plastic relaxation of stress (see supplementary information part B):

$$\frac{d}{dt}\sigma_{ab} = \mu[\partial_a V_b + \partial_b V_a - \delta_{ab}\,\gamma] - \tau_\sigma^{-1}\sigma_{ab} + \alpha \mathrm{m}_{ab}, \qquad (7)$$

where $\tau_\sigma^{-1}\sigma_{ab}$ accounts for stress relaxation with rate τ_σ^{-1}, while generation of stress by myosin is represented by $\alpha \mathrm{m}_{ab}$ – a term proportional to the myosin distribution denoted by m_{ab}. Because myosin is concentrated in filaments, any description of myosin stress generation must refer to their predominant orientation, hence myosin activity is best described by a tensor quantity, hence the subscripts on m_{ab}. In this generalized scenario, harmonic growth (for which the square bracket term is zero) would lead to a steady state with residual internal stress set by myosin activity: $\sigma_{ab}=\tau_\sigma \alpha \mathrm{m}_{ab}$.

Further, we have recently argued (Noll et al., 2017) that extension or contraction of cortical actomyosin bundles may act to respectively recruit or de-commission myosin in order to achieve

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the above balance between stress and myosin activity (σ_{ab}=$\tau_\sigma \alpha m_{ab}$), thus:

$$\frac{d}{dt} m_{ab} = \tau_m^{-1}(\sigma_{ab} - \tau_\sigma \alpha m_{ab}) \quad (8)$$

where τ_m^{-1} is the rate at which the myosin level responds to mechanical imbalance. Intuitively, mechanical imbalance in actomyosin bundles will lead to extension or contraction of the bundle (e.g. excess myosin would result in contraction) and our Eqn 8 corresponds to the hypothesis that myosin levels adapt to restore balance (e.g. reduce myosin level if the bundle is contracting). Conversely, pulling on a bundle would recruit more myosin until it balances the external tension. One can then show (by combining Eqns 7 and 8), that if the stress relaxation rate τ_σ^{-1} is high enough, myosin dynamics approximately follows:

$$\alpha \tau_m \frac{d}{dt} m_{ab} = \tau_\sigma \mu [\partial_a V_b + \partial_b V_a - \delta_{ab}\ \gamma], \quad (9)$$

with the cellular flow field V_a still determined by the mechanical equilibrium condition (Eqn 4). Rapid stress relaxation also means that it closely follows myosin, forcing $\sigma_{ab} \approx \tau_\sigma \alpha m_{ab}$. This intuitively plausible proportional relationship between local stress and myosin distributions (coarse grained on the scale of a few cells) is supported by experimental evidence (Streichan et al., 2017, preprint).

Remarkably, in Eqn 9 we recognize Eqn 2 with myosin tensor replacing the stress tensor. The conformal growth condition (when the right-hand side of Eqn 9 vanishes) thus maintains constant myosin level, and myosin level can serve as a proxy for mechanical stress and provide regulatory feedback coordinating growth.

The above analysis illustrates that mechanical feedback need not be limited to the simple elasticity model first used to introduce it. It works just as well in a model allowing for the active plasticity of the cytoskeleton, which can act as a mechanosensor and transducer of mechanical signals. It is also supported by experimental evidence, including observations that stretching tissues increases myosin (Kasza and Zallen, 2011), and growth-induced compression of epithelial cells is associated with reductions in myosin accumulation and cytoskeletal tension (Pan et al., 2016).

Influence of stress on growth in biological systems

Influence of stress on growth of cultured cells

We now consider some of the experimental observations that have revealed how cells actually respond to mechanical stress. The general inaccessibility of developing organs *in vivo* presents a challenge to direct examination of the impact of mechanical stresses. Conversely, cultured cell models, in which cells can be subjected to external manipulations *in vitro*, have provided important insights into how mechanical stresses influence growth. For many cell types, stretching stimulates cell proliferation, whereas compression inhibits it (Streichan et al., 2014; Eder et al., 2017; Legoff and Lecuit, 2015). Early experiments illustrating this included observations that stretching rodent skin or cultured cells could stimulate cell proliferation (Brunette, 1984; Curtis and Seehar, 1978; Lorber and Milobsky, 1968; Squier, 1980), and that compressing spheroids of tumor cells suppressed their growth (Helmlinger et al., 1997). By growing cultured cells on micropatterned substrates with distinct geometries, Nelson et al. (2005) were able to correlate patterns of mechanical stress with patterns of cell proliferation. The growth-suppressing effect of cellular compression, and growth-stimulating effect of increased tension, are consistent with the mechanical feedback hypothesis as they describe a change in growth rates that could reduce stresses associated with patterns of growth that are not conformal.

A characteristic feature of growth of many cells in culture is the suppression of proliferation at high cell densities, termed contact inhibition of proliferation (hereafter referred to simply as 'contact inhibition') (Holley and Kiernan, 1968; McClatchey and Yap, 2012), which, despite the name, depends on crowding not simply on cell contact (Kim et al., 2009; Puliafito et al., 2012; Streichan et al., 2014). Loss of contact inhibition is a hallmark of cancer cells (Ribatti, 2017). Contact inhibition in culture occurs because cells are spatially constrained and thus become more crowded regardless of the growth pattern. However, as discussed above, crowding could be generated *in vivo* by differential, non-harmonic growth. It is thought that multiple factors could contribute to contact inhibition, one of which is mechanical stress (McClatchey and Yap, 2012). Moreover, as discussed below, the Hippo signaling network, which is required for contact inhibition (Zhao et al., 2007), is a key mediator of biomechanical signals. Direct evidence for a contribution of compressive stress to contact inhibition has been provided by observations that stretching contact-inhibited cells can stimulate re-initiation of cell proliferation (Aragona et al., 2013; Streichan et al., 2014).

Growth of cells in culture requires a medium that includes growth factors that activate biochemical signaling pathways. The realization that growth is affected by both biochemical and biomechanical inputs raises the question of how these inputs are integrated to achieve normal growth rates. Studies in cultured cells have suggested a convergence on common processes. For example, at lower growth factor concentrations, contact inhibition occurs at lower cell densities, whereas at higher growth factor concentrations, contact inhibition occurs at higher cell densities (Brown et al., 1979; Kim et al., 2009). One key mechanism of integration is the existence of signal transduction pathways that respond to both biochemical and biomechanical cues; this is discussed further below.

Growth of developing organs *in vivo*

During development, cells must integrate a variety of biochemical cues that modulate organ growth. Some cues, such as nutrients and hormones that provide information about organismal nutrition and developmental stage, are systemic, and thus expected to promote relatively uniform growth. However, other cues are local, and may vary within a developing organ. One of the best-studied models of organ growth, which we shall use to discuss evidence for influences of mechanical stress on growth *in vivo*, is the developing *Drosophila* wing (Hariharan, 2015; Irvine and Harvey, 2015; Shingleton, 2010). The *Drosophila* wing forms from a sac of undifferentiated epithelial cells termed the wing imaginal disc, which grows during the larval stages from approximately 30-50 cells to 30,000-50,000 cells (Martín et al., 2009; Milan et al., 1996; Worley et al., 2013). If flies are starved, or insulin signaling is downregulated, then smaller flies emerge, with smaller, but normally shaped, wings, revealing an environmental regulation of organ size throughout the body (Hafen and Stocker, 2003). Wing growth also depends upon local growth factors. One of the most important of these is Decapentaplegic (Dpp; a bone morphogenetic protein family member), which is secreted by a stripe of cells along the middle of the developing wing disc, and then spreads out to more lateral cells (Fig. 3A) (Restrepo et al., 2014). Dpp is required for normal wing growth, increasing Dpp can increase growth, and Dpp pathway activity is graded from medial to lateral across the wing disc. However, for most of wing development, growth is evenly distributed throughout the wing disc (Fig. 3B) (Milan et al., 1996). A variety of

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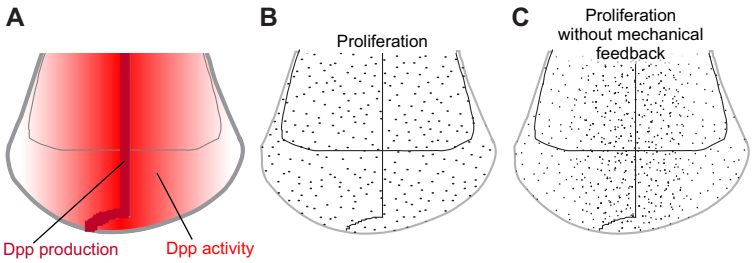

Fig. 3. Patterns of Dpp activity and cell proliferation in the wing disc. Schematics of part of the *Drosophila* wing imaginal disc. (A) The morphogen growth factor Dpp is produced from cells at a localized source along the center of the disc, and spreads out forming a concentration gradient. (B) Cell proliferation (shown by the dots) is essentially evenly distributed throughout the wing disc. Lines mark the compartment boundaries. (C) When Jub-mediated mechanical feedback is blocked (Pan et al., 2016) proliferation becomes unevenly distributed, with higher levels where Dpp signaling is higher.

models have been proposed and debated to explain how the gradient of Dpp pathway activity is converted into relatively uniform growth (Eder et al., 2017; Hariharan, 2015; Irvine and Harvey, 2015). These include proposals that cells can respond not simply to the levels of Dpp but also the gradient (Rogulja and Irvine, 2005), that cells respond to dynamic increases in Dpp concentration (Wartlick et al., 2011), and that responses are thresholded and there are intrinsic differences in response to Dpp in different regions of the disc (Restrepo et al., 2014). Another class of models have invoked an input from growth-generated mechanical stress onto local growth rates (Aegerter-Wilmsen et al., 2007, 2012; Hufnagel et al., 2007; Shraiman, 2005). It is likely that multiple mechanisms are involved in modulating the response to Dpp, but, as discussed below, recent evidence supports the notion of mechanical feedback as a contributing factor.

The Hippo signaling network integrates growth signals

The diverse growth regulatory cues that influence organ growth ultimately have to be integrated by cells. Although multiple mechanisms for this may exist, over the past decade the Hippo signaling network has emerged as a crucial integrator of both biochemical and biomechanical inputs into growth (Meng et al., 2016; Sun and Irvine, 2016). Hippo signaling is a conserved signal transduction network that was first discovered in *Drosophila* through the overgrowth phenotypes associated with mutations in pathway components (Bryant et al., 1988; Harvey et al., 2003; Jia et al., 2003; Justice et al., 1995; Kango-Singh et al., 2002; Lai et al., 2005; Pantalacci et al., 2003; Tapon et al., 2002; Udan et al., 2003; Wu et al., 2003; Xu et al., 1995). These overgrowths occur because of inappropriate activation of a transcriptional co-activator protein called Yorkie (Yki) (Fig. 4A) (Huang et al., 2005). Yki (or its mammalian homologs YAP1 and TAZ) is inhibited by protein kinases of the NDR family – Warts in *Drosophila*, and LATS1 and LATS2 in mammals. Most upstream components of Hippo signaling, including the Hippo kinase that gives the pathway its name, converge on regulation of Warts (Meng et al., 2016). Elevated Warts activity reduces Yki activity by promoting its exclusion from the nucleus and degradation. This reduces growth. Conversely, low Warts activity enables increased Yki activity and consequently increased growth. Studies of Yki regulation in different contexts have revealed that it can also be regulated by other families of protein kinases (Li et al., 2016; Mo et al., 2015; Taniguchi et al., 2015; Wang et al., 2015), and by cytoplasmic sequestration mechanisms that do not require phosphorylation (Badouel et al., 2009; Chan et al., 2011; Oh et al., 2009; Wang et al., 2011; Zhao et al., 2011).

The diverse inputs integrated by Hippo signaling include local patterning and cell contacts, organismal nutrition, and mechanical stress. Key upstream regulators of the pathway, such as Expanded, Merlin and angiomotins, localize to cell-cell junctions where they scaffold assembly of protein complexes that promote Warts activation, which provides a basis for Hippo pathway regulation by cell-cell contact and cell polarity (Sun and Irvine, 2016). Growth factors, such as EGF, that act through MAPK and phosphoinositide 3-kinase (PI3K) pathways can influence Hippo signaling (Fan et al., 2013; Reddy and Irvine, 2013), as can some G protein-coupled

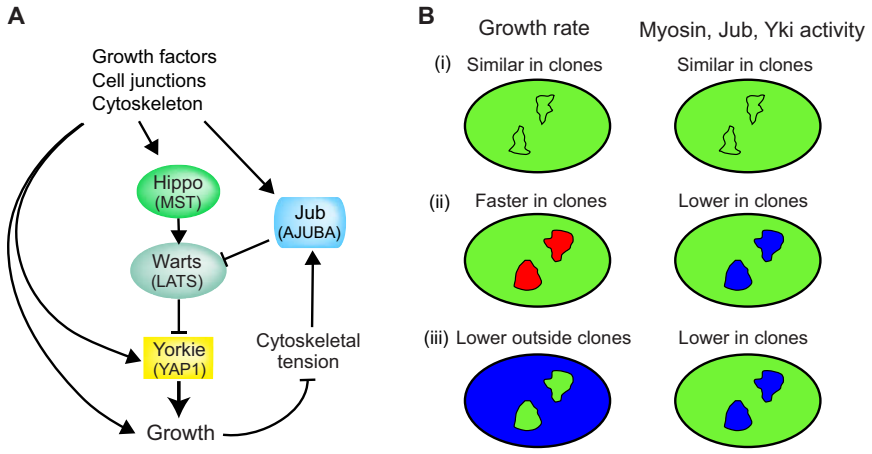

Fig. 4. Hippo signaling and mechanical feedback. (A) Simplified depiction of regulatory connections between some key components of the Hippo pathway, with *Drosophila* names above and vertebrate names of homologous proteins in parentheses below. (B) Schematics illustrating the consequences of differential growth rates in the wing disc epithelium on myosin levels (indicative of tension), junctional Jub levels, and Yki activity, with green indicating wild-type levels, red indicating higher levels, and blue indicating lower levels. (i) Marked clones of cells (outlined by thin black lines) growing at the same rate as surrounding tissue do not affect Myosin, Jub or Yki activity. (ii) Clones of cells growing at an abnormally fast rate and surrounded by tissue growing at a normal rate become compressed, leading to lower levels of Myosin, Jub and Yki activity. (iii) Clones of cells growing at a normal rate and surrounded by tissue growing at an abnormally slow rate also become compressed, leading to lower levels of Myosin, Jub and Yki activity. Adapted from Pan et al. (2016).

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receptor pathways that impinge on Rho (Yu et al., 2012). Multiple connections from nutritional and metabolic pathways, which link insulin signaling, mevalonate metabolism, AMPK activity, and TSC-mTor pathways to regulation of Yki activity have also been identified (Santinon et al., 2015). Cross-talk between Dpp signaling and Hippo signaling has also been identified, as transcription factors of these pathways can co-regulate downstream genes that promote growth (Oh and Irvine, 2011), and Dpp signaling influences the expression of genes, including *vestigial*, *dachsous* and *four-jointed*, that regulate Hippo signaling through the large cadherin Fat (Kim et al., 1996; Rogulja et al., 2008; Zecca and Struhl, 2010).

Biomechanical regulation of Yki

Yki activity is responsive to multiple aspects of cells' biomechanical environment, including flow-induced shear stress, tension sensed at integrin-ECM attachments, and tension sensed at adherens junctions (Dupont, 2016; Sun and Irvine, 2016). The mechanisms by which biomechanical signals are transduced appear to be complex, and are only partially understood. The stretch-sensitive calcium channel PIEZO1 responds to shear stress, and can promote YAP1 activity through unknown mechanisms (Pathak et al., 2014). Several proteins regulated downstream of integrin signaling have been shown to be able to influence YAP1 activity, including PI3K, FAK (PTK2) and SRC (Elbediwy et al., 2016; Kim and Gumbiner, 2015). Total levels of F-actin in a cell, which can be sensitive to the mechanical environment, impinge on Yki activity through still undefined mechanisms (Aragona et al., 2013; Fernández et al., 2011; Sansores-Garcia et al., 2011). Some of the regulation of YAP1/Yki through F-actin levels and integrin attachment sites (focal adhesions) is independent of Hippo signaling, and may be mediated through other kinases, such as SRC, or kinase-independent mechanisms. The spectrin cytoskeleton has also been found to influence Hippo signaling, although how it is connected to the Hippo pathway remains unclear (Deng et al., 2015; Fletcher et al., 2015; Wong et al., 2015). Tension at adherens junctions can also promote Yki activity (Benham-Pyle et al., 2015; Rauskolb et al., 2014), and at least in *Drosophila* this has been shown to depend upon tension-dependent formation of a complex between Warts and a Warts inhibitor called Jub, which associates with the adherens junction component α Catenin under tension (Rauskolb et al., 2014). Within epithelial cells, adherens junctions are ideally suited to relay mechanical stresses, as they experience tension through their connections to the actin cytoskeleton (Lecuit and Yap, 2015).

Mechanical feedback *in vivo*

The identification of a pathway that links mechanical stress to the growth-regulatory Hippo pathway in epithelial cells made it possible to begin examining contributions of mechanical feedback to modulation of organ growth *in vivo*. A variety of genetic manipulations that lead to differential growth of patches of cells within a developing wing disc visibly affect the Jub biomechanical pathway, as recruitment of Jub and Warts to adherens junctions is reduced within faster-growing regions (Fig. 4B), leading to lower levels of Yki activity (Pan et al., 2016). These effects of differential growth could be attributed to reduced cytoskeletal tension within relatively faster-growing, and hence more compressed, cells. As Yki promotes growth, this influence of differential growth on cytoskeletal tension, and components of the Hippo pathway regulated by tension, provides a mechanism for mechanical feedback.

Knowledge of this mechanical feedback pathway also made it possible to assess the consequences of impairing mechanical feedback during wing development. Genetic manipulations that increase growth rates in small patches of cells while suppressing or bypassing the ability of compressive stresses to downregulate Yki-dependent growth (hence suppressing mechanical feedback) can induce signs of cellular compression, including distortions of neighboring cells, and reduced myosin activity within the over-growing cells (Legoff et al., 2013; Mao et al., 2013; Pan et al., 2016). This suggests that this mechanical feedback normally has a role in preventing the accumulation of stresses that distort tissues. Preventing Jub-mediated mechanical feedback throughout the developing wing was found to lead to uneven patterns of cell proliferation in the wing disc, with higher levels of proliferation now observed where Dpp pathway activity is higher (Fig. 3C) (Pan et al., 2016). The observation that, without mechanical feedback, proliferation is higher where Dpp signaling is elevated is consistent with models proposing mechanical feedback as an explanation for why proliferation is normally uniform despite graded expression of Dpp (Aegerter-Wilmsen et al., 2007; Hufnagel et al., 2007; Shraiman, 2005).

As the wing disc grows, cells in the center of the developing wing become more compressed (Aegerter-Wilmsen et al., 2012; Legoff et al., 2013; Mao et al., 2013; Nienhaus et al., 2009). Careful observations of proliferation rates revealed a transient period during early wing disc development when distal and proximal cells appear similarly stressed, during which cell proliferation rates are higher near the center of the wing disc (Mao et al., 2013). However, as the disc grows, and cells in the center begin to appear more compressed, this proliferation differential is lost. These observations fit models in which higher growth factor signaling in the center of the wing disc transiently leads to higher proliferation rates, but this leads to cellular compression, which triggers mechanical feedback that equals out growth rates across the disc (Aegerter-Wilmsen et al., 2007; Hufnagel et al., 2007).

In addition to providing a mechanism for achieving harmonic growth, the hypothesis that compressive stress inhibits growth was put forward by Hufnagel et al. (2007) and Aegerter-Wilmsen et al. (2007) as a possible mechanism of size determination in developing imaginal discs. In the simple models described above, our 2D body can expand indefinitely, and a conformal growth constraint is employed to direct its form. However, if the expansion of the boundary of our body were to become constrained, then continued growth would lead to accumulation of compressive stress, slowing down growth, as suggested for cell density-dependent contact inhibition. Biologically, this might occur for a number of reasons: constraints provided by neighboring tissues, physical attachment of cells to extracellular matrix that impedes their mobility, or diminished growth rates of cells near the boundary. The suggestion that organ size could be modulated by mechanical feedback mechanisms is also consistent with observations of cell density-dependent growth in other *in vivo* systems, such as during zebrafish fin regeneration, which also depends upon F-actin levels and Yap1 activity (Mateus et al., 2015).

Duration of growth

Organ (and organism) size depends not only on the pattern and rate of growth, but also on the duration of growth. Recent studies have revealed that Hippo signaling also intersects with hormonal signaling that regulates the timing of developmental transitions and hence the duration of imaginal disc growth: Yki interacts with the ecdysone receptor co-activator Taiman (Zhang et al., 2015), and Yki modulates ecdysone levels by regulating transcription of the *Drosophila* relaxin-like hormone Dilp8 (Ilp8) (Boone et al., 2016). Dilp8 was identified in part because of its role in delaying metamorphosis in animals with damaged tissues, thus allowing them more time to regenerate (Colombani et al., 2012; Garelli et al., 2012).

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Conclusions

In this Review, we have attempted to address explicitly the overarching question implicit in D'Arcy Thompson's book – how is growth linked to form? Mechanical considerations arise naturally in this context. A thin elastic sheet with in-plane internal stress will deform, assuming a 3D shape that relaxes stress and minimizes mechanical energy. As this internal stress can be generated by non-uniform growth within a 2D tissue layer, mechanics provides a connection between growth and form. An elastic shell of finite thickness could, however, remain flat if its internal stress does not exceed the buckling threshold (Audoly and Pomeau, 2010) and some growing tissues remain planar: plant leaves for example, or the central part of the *Drosophila* wing imaginal disc on which our discussion was focused. The condition of planar stressless growth restricts growth profiles to the class of harmonic functions, which, although being strongly constrained, is still large enough to allow isotropically growing flat 2D tissue to take any planar shape. We argue that the most robust way to ensure growth that does not lead to accumulation of stress is to have a feedback inhibition of stress onto growth. Remarkably, examinations of how mechanical stress actually influences growth in biological systems as varied as *Drosophila* and cultured mammalian cells are entirely consistent with this view, and moreover have revealed a signaling network, the Hippo pathway, that responds to stress and integrates mechanical inputs with biochemical signals.

Although our focus has been on animal development, growth mechanics is equally important in plant morphogenesis (Coen et al., 2017). In fact, because plant cells are rigid and non-motile, one can expect considerations of continuum elasticity to be even more valid. The role of mechanics in plant development has received considerable attention, revealing among other things the presence of mechanical feedback on growth (albeit through different mechanisms) (Coen et al., 2004; Hervieux et al., 2016; Nakayama et al., 2012), and the important role of controlled growth anisotropy in defining form (Coen et al., 2004). We also noted that plant leaves have provided an example of non-uniform, conformal growth, and it will be interesting to explore which other examples of non-uniform growth are conformal, and whether any non-harmonicity of their growth profile correlates with morphogenetic buckling.

Although we have focused our analysis on 2D growth, the mechanical considerations discussed easily extend into 3D growth, even though the convenient mathematical machinery of 2D conformal mappings, does not. However, we would expect the cell biological mechanisms that relay stress to growth regulatory pathways in multilayered tissues to differ from the Jub-Warts-Yki pathway discussed above, as adherens junctions respond to stress within the plane of the tissue.

Finally, we note that, despite exciting progress over recent years, there remain many unanswered questions involving both understanding the mechanics of stress generation and relaxation in growing tissue, and the role and detailed mechanism of mechanical regulation of growth. The ultimate question of how tissue and body shape is actually encoded in the 'executable program' of development is still open.

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Competing interests

The authors declare no competing or financial interests.

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Supplementary information

Supplementary information available online at
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