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Physical control of tissue morphogenesis across scales

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Abstract

During embryogenesis, tissues and organs are progressively shaped into their functional morphologies. While the information about tissue and organ shape is encoded genetically, the sculpting of embryonic structures in the 3D space is ultimately a physical process. The control of physical quantities involved in tissue morphogenesis originates at cellular and subcellular scales, but it is their emergent behavior at supracellular scales that guides morphogenetic events. In this review, we highlight the physical quantities that can be spatiotemporally tuned at supracellular scales to sculpt tissues and organs during embryonic development of animal species, and connect them to their cellular and molecular origins.

Introduction

From the branching geometry of lung or kidneys to the structure of limbs and digits, tissue and organ morphology is intimately related to proper organ function and, therefore, to the survival of the organism. A myriad of works over the past several decades have revealed a critical role of signaling molecules in orchestrating cellular events during tissue and organ morphogenesis[1]. However, despite their key role in developmental processes, even a detailed knowledge of the signaling molecules and their connections to cellular events cannot, per se, provide a complete understanding of morphogenetic events during development. Tissues and organs are also physical objects (materials) that are sculpted in the 3D space during embryonic development, as highlighted by D'Arcy Thompson a century ago[2] and generally acknowledged today. While the current knowledge of the molecular control of morphogenesis dwarfs our understanding of how embryonic structures are

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physically built, the development of new techniques to quantify mechanics within living embryos[3] and the advent of interdisciplinary approaches[4], have sparked new and rapidly increasing interest in the physical aspects of embryonic development.

From a physical perspective, embryonic tissues are complex, active materials, with the ability to self-shape, remodel and, in some cases, even self-heal or regenerate. Regardless of their complexity, any material is subject to fundamental physical laws that constrain how mechanical forces propagate in the structure and how addition of new material (e.g., via cell proliferation) is spatiotemporally redistributed. However, there are several physical quantities that remain largely unconstrained by physical law and can be genetically controlled and spatiotemporally modulated by cells to sculpt tissues into virtually any desired shape. Similar to sculpting inert materials (e.g., clay molding or even 3D printing), shaping embryonic tissues and organs requires fine spatiotemporal control of several physical quantities to attain the desired functional morphology.

Inspection of the fundamental physics governing material (tissue) morphogenesis highlights three physical quantities that can be modulated and, therefore, can be used to shape tissues and organs: **volumetric growth**, **tissue material properties** and **active forces** (Fig. 1A; Box 1). Both inhomogeneities and anisotropies (Box 1) in any of these quantities, or combinations of them, can be employed to sculpt the desired shape. For instance, the spatiotemporal control of a growth zone (proliferation zone) in the frontonasal mass of developing bird beaks has been shown to directly affect the adult beak shape in several avian species (Fig. 1B). While a narrow proliferation zone gives rise to the slender chicken beak, widening the proliferation zone widens the beak into a bill, as observed in ducks[5]. More strikingly, offsetting the proliferation zone posteriorly and dorsally bends the tissue, making the curved cockatoos beak[6]. Tight spatiotemporal control of apoptotic tissue regions (negative growth or loss of tissue) via programmed cell death can also be used to shape tissues and it is an essential mechanism of digit formation in vertebrates[7].

In contrast to the abovementioned examples, tissues can be shaped in the absence of growth. For instance, during ventral furrow formation in Drosophila, the regional control of active forces in the tissue via spatially-graded actomyosin activity, drives the tissue invagination necessary for gastrulation[8] (reviewed in[9]) (Fig. 1B). Beyond active forces, direct *in vivo* and *in situ* measurements of tissue mechanics during posterior axis elongation in zebrafish have recently revealed the existence of a fluid-to-solid transition in the tissue state that guides body elongation[10] (Fig. 1B). While the posterior-most elongating tissue is maintained in a fluid-like state, enabling tissue remodeling and changes in shape, the tissue progressively transits into a solid-like state as it moves anteriorly, establishing tissue architecture and mechanically supporting body elongation[10].

Below we discuss the cellular and molecular processes that affect each of these physical quantities during tissue morphogenesis, as well as the interplay (or coupling) between them.

Volumetric growth

While uniform, isotropic volumetric growth causes the size of a tissue or organ to change, inhomogeneous growth or anisotropic growth can both lead to shape changes (Box 1). A number of cellular processes can lead to tissue volumetric growth, including cell proliferation or apoptosis (negative growth), changes in cell size, and deposition/degradation of extracellular matrix (ECM).

Cell proliferation/apoptosis is the most studied type of volumetric growth. In many embryonic tissues, cell proliferation is inhomogeneous, with some highly proliferative domains and regions with little proliferation which, as described above, can drive important morphogenetic changes (Fig. 1B). Several signaling molecules, such as BMP4 in beak development[5] or FGF, RA and BMP signaling in digit formation[11], have been shown to control the spatial localization of cell proliferation/apoptosis (Fig. 2; Table 1). Beyond the regional control of proliferation and/or programmed cell death, oriented cell divisions along specific spatial directions (anisotropic growth) are also known to contribute to tissue shape changes[12], as observed during germ band extension and wing imaginal disc morphogenesis in Drosophila[13,14], or during zebrafish gastrulation[15].

Secretion of extracellular matrix (ECM) by cells can considerably contribute to volumetric growth in specialized tissues, such as in cartilage, tendon or bone[16,17]. In contrast, in the less specialized tissues, such as those found at earlier developmental stages, ECM deposition does not strongly contribute to the volumetric growth of the tissue, but rather affects the tissue material properties (see below) and provides biochemical and biophysical cues for cells[17,18].

Although growth contributes to shaping tissues, in most cases it is not possible to explain morphogenetic events solely from volumetric growth, as has been shown, for instance, in limb morphogenesis[19,20]. Typically, spatiotemporal modulations in tissue material properties or active forces must occur in conjunction with growth to shape functional structures.

Active forces

Perhaps the most studied physical quantity in relation to morphogenesis is mechanical force and, more specifically, active cellular forces. Several cellular processes and structures can generate active forces and affect tissue form, including actomyosin contractility and cell volume changes.

The control of regions in the tissue with high actomyosin activity has been shown to be key for gastrulation movements in the fly embryo. During ventral furrow formation, a region of the tissue defined by the expression of Twist and Snail displays increased and pulsed actomyosin constriction that initiates the invagination of the tissue and gastrulation movements[8,21,22] (Fig. 1B). The large-scale spatial distribution of myosin and its anisotropy in the tissue are thought to drive global tissue morphogenetic flows during gastrulation[23,24]. Also in Drosophila, germband elongation has been shown to involve planar-polarized (anisotropic) distributions of actomyosin contractility[25]. Myosin is more

strongly localized at junctions oriented along the dorso-ventral direction, and less strongly localized at anterior-posterior junctions[26–28]; this anisotropy in myosin localization and in myosin flows within the cell[29] result in local anisotropic forces[28,30] that cause polarized cell intercalation, generating convergent extension movements and axis elongation[28,31,32]. Anisotropic force generation is also essential to morphogenetic events in vertebrates, including gastrulation movements[33] and early body elongation[34,35], where planar cell polarity controls force anisotropy during convergent extension[36]. Recent quantitative measurements of supracellular forces during zebrafish posterior axis elongation revealed a posterior-to-anterior increase in actively-generated mediolateral (anisotropic) forces associated to the thinning of the body axis, but not to its elongation[10]. Finally, recent experiments showed that tissue morphology in medaka *hir* mutants is strongly affected by gravity[37]. These defects appeared to be a result of reduced actomyosin contractility leading to a failure to correctly assemble fibronectin fibrils, presumably leading to reduced tissue tension and the flattening of the body.

Several of the abovementioned processes require the transmission and coordination of active forces across multiple cells. This coordination relies on the formation of supracellular actin cables that physically connect the cytoskeleton (and especially the cortices) of multiple cells through cell-cell adhesion proteins, such as E-cadherin, and connectivity between adhesions and the actin cytoskeleton via α-catenin and β-catenin[38–42] (Fig. 2; Table 1).

Changes in cell volume driven by osmotic changes can also generate constrictive forces in a tissue that can drive global tissue shape changes. An example of this is the caspase-mediated cell volume decrease seen in the *Drosophila* amnioserosa during dorsal closure. The collective cell shrinkage produces a contractile force, that works with the supracellular actin cable at the leading edge of the dorsal epithelium to close the epithelium over the dorsal surface of the embryo[43].

Tissue material properties

While active forces power cell movements, the material properties of the tissue define the morphogenetic movements that result from both active and passive forces in the tissue. There are several cellular processes and structures that impact the tissue material properties, including cortical actomyosin contractility, cell-cell or cell-matrix adhesion or ECM physicochemical state.

In tissues with little to no ECM between cells (except at tissue boundaries), tissue material properties depend strongly on the supracellular tissue architecture (Fig. 2), which is largely controlled both by the mechanics of cell-cell contacts, as well as on cellular processes like cell rearrangements and divisions[12,40]. Measurements in tissue explants from amphibian embryos have shown that different tissues are characterized by different elastic and viscous properties[44,45] and that axial tissues during body elongation display an actomyosin-dependent temporal stiffening[45], which is thought to help increase the tissue mechanical integrity and maintain tissue architecture as development proceeds[45]. In addition, recent direct *in vivo* measurements of tissue material properties during zebrafish body axis elongation show the existence of an anteroposterior, N-cadherin-dependent gradient in tissue

viscoelasticity[46]. Since the material properties of the cellular microenvironment are known to strongly affect cell behavior, it is possible that spatial variations in tissue material properties act as differential biophysical cues. Indeed, recent experiments in amphibian embryos have revealed that head mesoderm stiffening triggers the collective migration of neural crest cells and coordinates key morphogenetic events, namely gastrulation movements and neural crest migration[47]. Other *in vivo* measurements of mechanical properties have focused on the mechanics at the cellular and subcellular scales[30,48] and revealed the material properties of cell-cell contacts directly. Recent experiments indicate that the viscoelastic dissipation at cell-cell contacts may stabilize the cell shape changes necessary for tissue morphogenesis during germ band extension in Drosophila[49].

More recently, comprehensive measurements of both forces and tissue material properties *in vivo* have revealed a fluid-to-solid tissue transition that guides posterior body axis elongation in zebrafish[10]. Posterior tissues were shown to display a less constrained cellular microenvironment (more extracellular spaces) and higher cell-cell contact active fluctuations, driving cellular rearrangements and effectively ‘melting’ the tissue into a fluid-like state (plastic behavior; Box 1). After remodeling at the posterior end of the body, tissues progressively move anteriorly and turn solid-like through a jamming transition caused by increasing cellular confinement and smaller cell-cell contact active fluctuations. The solid-like tissue state helps establish tissue architecture and mechanically supports the posterior extension of fluid-like tissues[10]. Cellular movements observed in chicken embryos during axis elongation[50], which are under the control of FGF signaling, are consistent with the physical mechanical of axis elongation reported in zebrafish embryos. In line with these observations, recent experiments suggest that cellular jamming also occurs during Drosophila gastrulation in epithelial tissues[51], affecting cell shapes and potentially restricting morphogenetic movements.

Spatiotemporal variations in matrix deposition or remodeling have been shown to affect morphogenetic events. For instance, branching morphogenesis is strongly dependent on proper fibronectin deposition for cleft formation[52] and also on the controlled spatiotemporal remodeling of the basement membrane[53]. While direct in vivo and in situ measurements of ECM mechanical properties within developing 3D tissues have never been achieved, it is thought that spatial variations in ECM assembly/remodeling can lead to spatiotemporal variations in its mechanical properties, thereby affecting morphogenesis[54]. Recent quantitative experiments have shown that the biased deposition of basement membrane during Drosophila oogenesis leads to anisotropic and inhomogeneous matrix mechanical properties, which constrict growth of the egg chamber medially and bias growth along the antero-posterior axis[55,56].

Interplay between physical quantities

Since the same molecular and cellular structures can affect multiple physical quantities, changes in a particular molecule or structure can lead to simultaneous changes in different physical quantities (Fig. 2). Indeed, changes in myosin II activity affecting cortical contractility can affect both active force generation and the tissue material properties[45]. More generally, the three physical quantities described above can be coupled, meaning that

changes in one quantity may lead to concomitant changes of another one. The interplay (or coupling) between physical quantities is essential to morphogenetic events, as highlighted in several key developmental processes.

Interplay between mechanical forces and cell proliferation.

When cells proliferate in a confined environment, the buildup of isotropic stresses can affect cell proliferation and anisotropic stresses can affect the orientation of cell division[12,57]. For instance, during the development of the *Drosophila* wing imaginal disc, cells in the center of the wing pouch are thought to be compressed and barely proliferate, whereas cells in the periphery experience circumferential tension and proliferate more, dividing along the directions of maximal tension1[57,58]. At pupal stages, a large scale tissue contraction subjects epithelial cells in the wing blade to anisotropic tension, which orients cells divisions (and coordinates other cell behaviors) to elongate the wing proximo-distally to properly shape it[59]. In the Drosophila dorsal thorax, the inhomogeneous distributions of anisotropic stresses have also been shown correlate with the spatial distribution of cell division orientation[60], with local cell shapes and strains affecting the cell division axis[61]. In zebrafish, cells of the enveloping cell layer divide along the direction of maximal tension during epiboly, thereby reducing the anisotropic stress in the tissue and aiding tissue movement toward the vegetal pole[62]. In mouse embryos, tension anisotropy in the limb ectoderm results in oriented cell divisions that facilitate cellular rearrangements and limb bud outgrowth[63].

Cell proliferation can also affect mechanics and morphogenesis. Recent experiments showed that spatially uniform cell proliferation can drive a mechanical instability causing the simultaneous formation of branches in the developing murine airway epithelium[64,65]. Moreover, differential proliferation in adjacent, physically connected tissues, has also been shown to drive a mechanical instability causing the looping pattern in the avian gut[66].

Interplay between mechanical forces and tissue material properties.

Active cell-scale forces have been recently shown to be necessary to fluidize embryonic tissues that would otherwise be in a solid-like state, directly relating cell-generated forces to tissue material properties[10]. Mechanical forces can both promote the secretion of new ECM or align previously established ECM, thereby affecting the tissue material properties and their anisotropy. During the formation of the heart in zebrafish, shear stress generated by blood flow promotes the synthesis of fibronectin1b via activation of the transcription factor *klf2,* resulting in the reorganization of cells in the heart tube and the proper development of the atrioventricular valve[67]. In tooth development, odontogenic differentiation is triggered by physical compaction of the mesenchyme (mesenchymal condensation), a process which results in the deposition of collagen VI which stabilizes the forming odontogenic stem cell niche[68,69]. Beyond the mechanical stimulation of matrix deposition, forces generated by cells in the ECM can orient the matrix fibers and lead to anisotropic mechanical properties. Recent *in vitro* experiments show that active cellular pulling on the ECM reorganizes the matrix, leading to the alignment of matrix fibers[70,71] that generates anisotropic mechanical properties which help define the direction of collective cell migration[72]. Finally, it has recently been shown that the interplay between tissue stiffness and active force

generation in adjacent tissue layers specifies the follicle pattern in the developing avian skin[73].

The couplings between physical quantities themselves and with signaling events contain essential information to gain a holistic understanding of tissue morphogenesis. With the development of new techniques to directly probe physical quantities *in vivo*[3], it is becoming possible to quantitatively study the molecular control of physical quantities and how these physical cues affect genetic programs during embryonic development.

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Box 1

Three physical quantities can be spatiotemporally tuned to guide tissue morphogenesis:

Volumetric growth –

the change in volume of a tissue (including negative growth, or shrinkage), generally involving exchange of matter with the environment. Such changes can be due to cell proliferation or programmed cell death, but also due to cell volume changes or extracellular matrix deposition.

Active forces –

forces generated by molecular and cellular mechanisms that consume energy (ATP or GTP-consuming processes), such as actomyosin contraction or polymerization of actin filament networks or microtubules. These forces are to be differentiated from passive forces that are not directly generated by cellular processes that employ energy consumption. These include elastic and dissipative forces (shear, crowding pressure, etc.). Mechanical stresses, defined as force per unit surface, can be active or passive following the definition detailed above for forces.

Tissue material properties –

The material (or mechanical) properties of a material, including living tissues, dictate how the material deforms or flows in response to mechanical forces. At supracellular, tissue scales, the mechanical properties not only depend on cellular structures (cortical tension or adhesion levels) but also on the local tissue architecture and extracellular structures (such as matrix). Tissue mechanical parameters include its stiffness (resistance to deformation), viscosity (resistance to flow), or viscoelasticity (time-dependent elastic/viscous behavior), but also its plastic behavior, characterized by a yield stress, which quantifies the minimal mechanical stress needed to make the tissue flow like a fluid: below the yield stress, the tissue behaves like a solid and above like a fluid.

Any of these physical quantities can be isotropic or anisotropic and homogeneous or inhomogeneous, which are defined as:

Isotropic, anisotropic, homogenous and inhomogeneous physical quantities –

A physical quantity is isotropic if its properties are equal along all spatial orientations. Anisotropic physical quantities display differences along different spatial orientations. A physical quantity is homogenous if it is spatially uniform, i.e. if its magnitude does not change with spatial position. In contrast, inhomogeneous physical quantities vary from point to point in space, displaying differential variations in their magnitude. For instance, cell proliferation could be anisotropic (cells dividing along a specific spatial orientation, e.g., along an embryonic axis) and homogeneous (the division rate being the same at every point of the tissue). In contrast, cell proliferation could be isotropic (the cell division axis being randomly oriented) and inhomogeneous (the proliferation rate changing with location in the tissue).

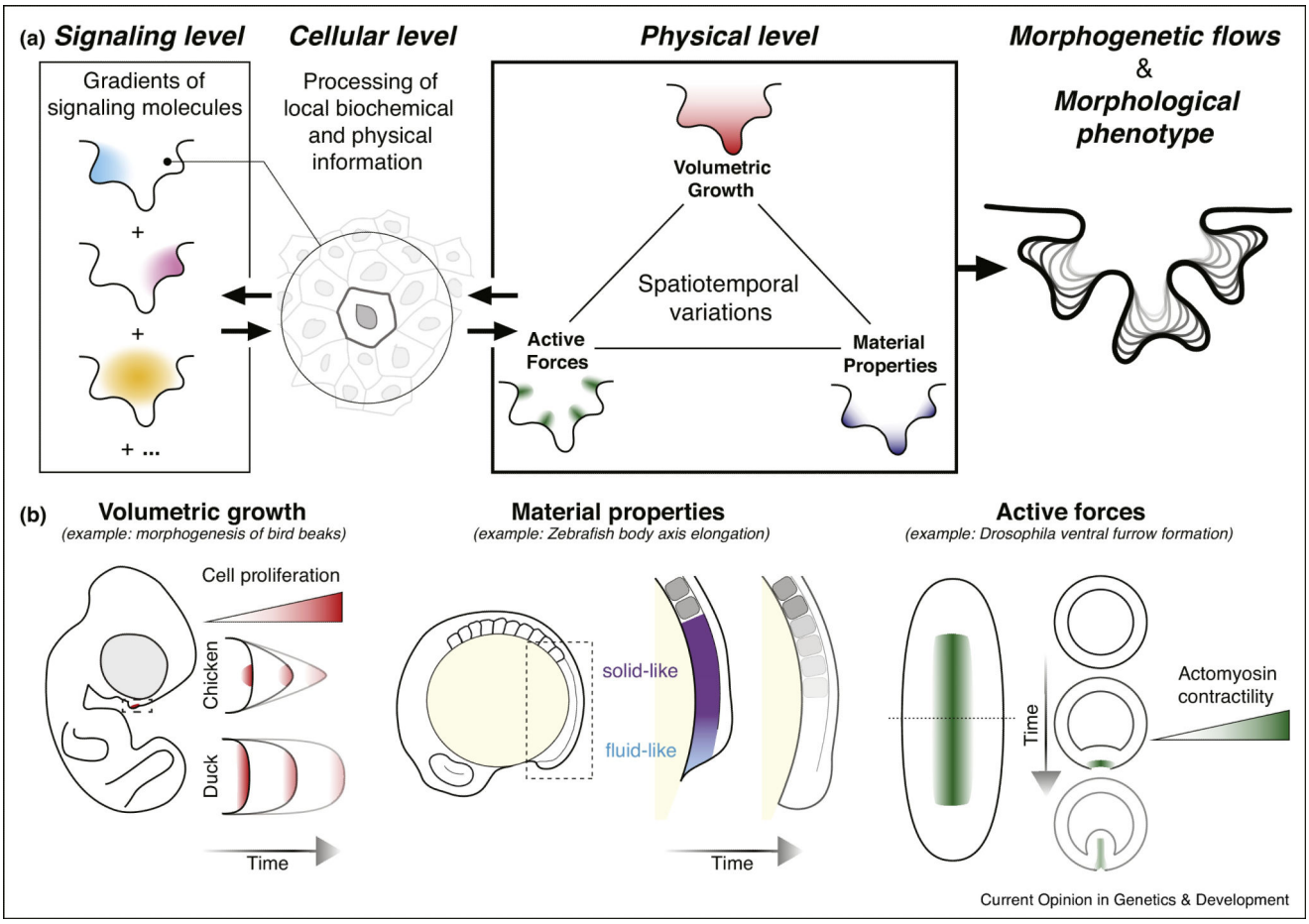

Figure 1 –. Sculpting tissues via spatiotemporal variations of key physical quantities.

A. The functional, morphological phenotype is ultimately achieved by controlling in space and time three physical quantities (physical level): volumetric growth, tissue material properties and active forces. Such control is, at least partially, due to gradients in signaling molecules in the tissue (signaling level). Cells within the tissue both sense and respond to local values of biochemical and physical cues (cellular level). **B.** Examples of morphogenetic processes for which spatiotemporal control of specific physical quantities have been identified. A localized cell proliferation zone (controlled region of volumetric growth) is essential to shaping bird beaks. Changes in the shape of the proliferation zone change the shape of the resulting beak, as illustrated for chicken and duck (dorsal views of developing beak) [5,6] (left). A fluid-to-solid transition between tissue physical states along the anteroposterior axis guides posterior body elongation in zebrafish[10]. In this case, the spatiotemporal control of fluid-like and solid-like tissue regions shapes the tissue (middle). In *Drosophila*, high levels of actomyosin contractility are restricted to a ventral region to enable invagination of the ventral furrow[9] (right). Dotted line indicates location of transverse cross sections shown on right.

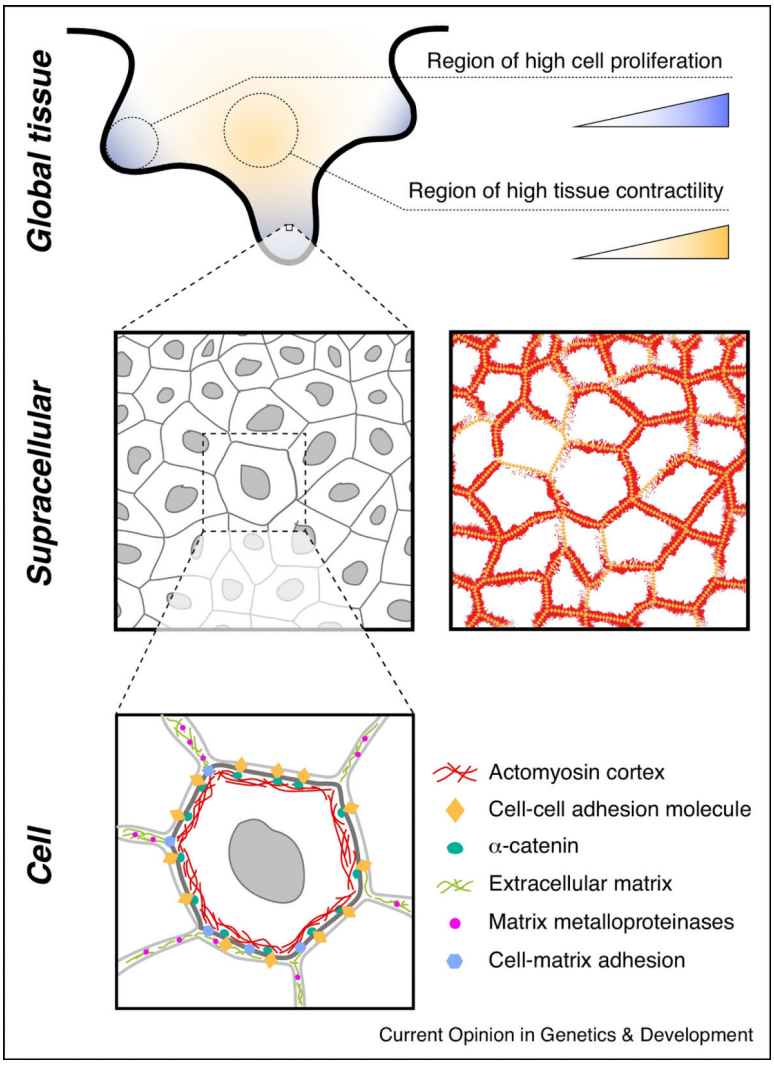

Figure 2 –. Control of physical quantities across scales.

The control of physical quantities at different scales (global tissue scale; supracellular scale; cell/subcellular scales) involves many molecular players and structures (Table 1). Multiple physical quantities are controlled simultaneously for coordinated morphogenesis. *Global tissue scale*: A tissue may contain gradients of growth (illustrated here by cell proliferation), active forces (illustrated here by tissue contractility), and material properties. These global gradients of physical parameters are defined by gradients of signaling molecules and signaling pathway activity. *Supracellular scale*: Emergent collective behavior arising from the interactions of cells in the tissue defines physical quantities at the supracellular scale. Therefore, physical quantities at these scales depend on the local tissue architecture, as well as the molecules involved in maintaining physical interactions between cells (e.g., connection of cytoskeletal structures across cells: adhesion complexes (orange diamonds), α-catenin and β-catenin, cortical actomyosin (red), etc.). *Cell scale*: Several cellular structures and molecules control cell mechanics. Active forces can be generated at the cell cortex via actomyosin contractility, via actin polymerization at the cell surface, via osmotic

pressure changes, etc. Changes in physical quantities at one scale typically affect other scales in the tissue, leading to coordinated development and morphogenesis.

Table 1:

Biological control of physical quantities across scales

	Physical Quantity		
Scale	**Volumetric Growth**	**Active Forces**	**Material Properties**
Global tissue	• Spatial inhomogeneities in cell proliferation controlled by gradients in signaling molecules (BMP4, RA, FGF, etc.; see e.g.[5]) • Global tissue mechanical forces affect both orientation (anisotropy) and rate of cell proliferation (see e.g.[58,62]).	• Spatial inhomogeneities in active forces controlled by transcription factors spatial localization (see e.g., {Heer:2017hm, Leptin:1990ub}) • Force anisotropy controlled by planar cell polarity (see e.g., {Zallen:2004wg, Rauzi:2008gz, Wallingford:2000c})	• Unknown signaling control.
Supracellular	• Cell proliferation/apopt osis. Cell shape and local forces affect both orientation and rate of cell division (see e.g., [60,61]). • Extracellular matrix deposition (see e.g.{Kalson:2015ez,Rozario: 2010fz}).	• Supracellular actomyosin networks(see e.g. [21,40,41]) • Supracellular forces depend on cell adhesion and the connection of cell cortex and adhesion complexes (e.g. α-catenin) (see e.g. {Mongera: 2018wv,Vasquez:2016dy,Lecuit: 2015hd,Lecuit:2011ec }) • Collective cell migration[74]	• Supracellular mechanical properties depend on cortical tensions (seee.g. {Heisenberg:2013tla, Lecuit:2007cw, Zhou: 2009hz}), cell-cell or cell-matrix adhesion{Mongera:2018 wv, Serwane: 2017ht}, extracellular matrix properties and remodeling (via matrix metalloproteinases{Bon nans:2014kn}), cell-cell rearrangements and extracellular spaces{Mongera:2018wv}, etc. {Khalilgharibi:2016c z, Campas:2016gd}.
Cell/subcellular	•Molecularregulators of cell growth, shrinkage, proliferation and apoptosis control cell and local tissue growth (see e.g., [43,75,76]).	• Acto-myosincontractility (e.g. cortical tension[40,41]) • Osmotic pressure changes (through ion channels[43]) • Actin polymerization, especially during collective migration (e.g. formation of filopodia and lamellipodia[74]).	• Intracellular cytoskeletal structures{Khalilgharibi :2016cz}, especially the cell cortex. Adhesion molecules. Connection of actin cortex to adhesion molecules {Vasquez:2016dy, Lecuit:2015hd}. • Force generating molecules, especially non-muscle myosin II force generation at the cell cortex[40,41]. • Secretion of extracellular matrix components and matrix metalloproteinases[17,77].