**The dynamics and biophysics of shape formation: common themes in plant and animal morphogenesis**

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eTOC: Comparing plant and animal morphogenesis reveals underlying principles used to change cell growth and contraction, which shapes the tissue. The principles discussed in this review include coordination of gene expression, signaling, growth, contraction, and mechanical and geometric feedback**.**

Abstract

The emergence of tissue form in multicellular organisms results from the complex interplay between genetics and physics. In both plants and animals, cells must act in concert to pattern their behaviors. Our understanding of the factors sculpting multicellular form has increased dramatically in the past few decades. From this work, common themes have emerged that connect plant and animal morphogenesis, an exciting connection that solidifies our understanding of the developmental basis of multicellular life. In this Review we will discuss the themes and the underlying principles that connect plant and animal morphogenesis including

the coordination of gene expression, signaling, growth, contraction, and mechanical and geometric feedback**.**

## Introduction

Morphogenesis is an inherently mechanical process that is dictated to a large extent by the mechanical properties of cells. Plant and animal cells have divergent systems that regulate their mechanics. Plant cell mechanics are dominated by their cell walls, which are composed of cellulose fibers, pectin, and hemicellulose whose directionality, density, and remodeling rate determine extensibility[1-4]. The plant cell wall surrounds the entire cell, facilitating connections with all neighboring cells. The directionality, density, and remodeling of the contractile actomyosin cytoskeleton determines the mechanics of animal cells[5]. A plethora of cell junction types connect animal cells and couple their cortical cytoskeletons. These junction types have different molecular components and are localized to different subcellular compartments, such as the apical adherens junctions in epithelia. While the cortical cytoskeleton does not directly contribute to plant cell mechanics, cortical microtubules do serve as tracks for cellulose synthesis and thus microtubule orientation is responsible for directing the orientation of cellulose fibers[6]. Plant cell growth results from the balance between turgor pressure and cell wall extensibility – turgor pressure promotes growth and the cell wall resists it[1; 3; 4]. Animal cell contractility results from the balance between external tension, hydrostatic (internal) pressure and cortical tension exerted by the actomyosin cytoskeleton.

The stunning and recognizable aerial shapes of flowering plants emerge from cell growth and division giving rise to the repeated patterns of leaves, branches, stems, and flowers. Meristems, the growing tips of the plant, are responsible for initiating both leaves and floral organs[7]. The shoot apical meristem has a stem cell niche in the center. As the cells in the niche grow and divide, they are displaced out of the niche toward the periphery and become competent to form organs. The organs initiate as a group of cells that bulge out of the meristem

periphery, and these early-stage organs are referred to as primordia. Differential growth between cells in initiating organ primordia and the surrounding boundary cells leads to the emergence (i.e., evagination) of a rounded primordium. Organ primordia emerge from the meristem in a stereotypic pattern (a.k.a. phyllotaxis) that is dictated by the plant species and organ type. Subsequent differential growth rates, polarity of growth, and divisions of cells within the developing organ gives rise to the complex three-dimensional forms of the leaves, sepals, petals, stamens, and carpels[8-10].

Animal organ shapes and their diversity are equally complex. While animal cell growth is a key morphogenetic mechanism, this review will focus on animal cell contractility in cell layers. Contractility polarized to one side (e.g. apical) of an epithelial sheet can result in inward bending due to shrinkage of one surface (i.e. apical) relative to another (i.e. basal) connected surface, similar to how a bimetallic strip coils when one metal changes in length more than the other[11]. Similarly, differential growth and/or contractility in adjacent layers of cells can induce invaginations or tissue bending, which is relevant to morphogenesis in both plants and animals[12; 13]. We focus on differential surface contractility in animal cells promoting invagination because one can think of this as the inverse of plant organ primordia emergence. For example, plant organ primordia emergence results from growth-dependent protrusion, whereas animal cell invagination can result from shrinkage (i.e. contraction)-dependent invagination.

In this article, we will provide a brief review of some of the molecular mechanisms and physical mechanics involved in plant and animal development – at the level to engage researchers in plant/animal morphogenesis with the other's system and major questions. This will not be a comprehensive review of plant or animal morphogenesis and we suggest other excellent reviews on each of these topics[4; 9; 14-17]. One key difference that we will not discuss is the presence of migratory/mesenchymal cells in animals, but not plants. Instead, we focus on themes that will highlight similarities in concepts between plant and animal morphogenesis. One theme will be how combinations of transcription factors and signaling molecules set up patterns

that control tissue shape. A second theme will be how oriented growth and cell division give rise to three-dimensional shape. A third theme will be how mechanical feedback between cells can reinforce these patterns and make the process more robust. Finally, a fourth theme will be the importance of cell dynamics and heterogeneity in robustly sculpting the overall tissue.

***Patterning sets up organ shape through differential growth and contraction***

During morphogenesis, tissues undergo extensive remodeling events that transform them from a simple, sometimes two-dimensional, collection of cells into complex, three-dimensional structures. Such global rearrangements depend on the spatial and temporal coordination of individual cell fates, shapes, mechanics, and movement or displacement. Precise patterning and tissue sculpting can be achieved by signals that define specific zones within a tissue through the activation of signaling pathways that culminate in patterns of cell behaviors[18]. Depending on morphogen signal level and timing, there can be different responses to the same signal. Classically, morphogens are signaling molecules whose expression patterns occur as gradients and whose effects depend on concentration[19; 20]. The plant epidermis and animal epithelia are both sheets of cells that are physically connected by their cell wall or junctions, respectively. The juxtaposition of signaling factors that cause different cell behaviors in a developing tissue create differences in curvature that shapes the organ. In plants, the specification of zones of fast growth adjacent to slow growth generates curvature. In the context of this review we refer to growth as the addition and/or redistribution of mass/volume in the plane of a tissue. Differential growth in plant tissues can be thought of as being analogous to differential contractility in animal development, but with opposite direction of curvature. Key to the location and timing of growth or contraction is the concentration and duration of signals from regulatory factors.

*Plants: Signaling gradients establish differential growth to enable organ emergence*

Plant organ primordia emerge from the meristem because the organ cells grow faster than the surrounding slow growing boundary cells and the undifferentiated meristem cells[21-23]. This zone of fast growth surrounded by slow growth creates an “areal conflict” that generates a rounded bulge from the surrounding tissue[24-26]. Plant hormone signaling and transcription factors pattern these zones of fast growth surrounded by slow growth and thereby dictate the timing and location of organ initiation within the meristem (Figure 1A-B). Specifically, maxima of the plant hormone auxin determine the location of organs before they emerge[27]. Auxin response promotes cell wall extensibility and cell growth[28; 29]. Meanwhile the transcription factors CUC1, 2 and 3 are expressed in the boundaries around organs and suppress growth (Figure 1A-C)[30-33]. Organ initiation cannot occur in the absence of auxin maxima[34] and CUC loss of function can cause fused organs[35]. Therefore, the juxtaposition of fast and slow growth is necessary to initiate distinct organ primordia.

Plant meristems continually make primordia throughout the life of the plant. Therefore, auxin concentration within the meristem is dynamic, forming maxima in different locations within the meristem as the plant grows (Figure 1B). The locations of these maxima within the meristem follow a pattern that results in a defined timing and arrangement of organs around the stem, or phyllotaxy. The Arabidopsis mutant drmy1 has diffuse auxin localization in the floral meristem rather than distinct maxima, which alters timing and position of organ initiation[22]. PIN-FORMED 1 (PIN1) is an auxin efflux carrier that is necessary for transporting auxin to create auxin maxima, and *pin1* mutants have “pin-shaped” meristems due to their inability to initiate organs[36]. Imaging data has demonstrated that PIN1 creates auxin maxima by polarizing to the cell membrane facing the location of the next primordia, which pumps auxin up its concentration gradient[27]. Mathematical modeling has postulated that there is positive feedback between high auxin and PIN1 (Figure 1A). In simple models, PIN1 polarizes towards neighboring cells with the highest auxin concentration, which causes auxin to be pumped up its concentration gradient. This positive feedback is sufficient to create auxin maxima in the correct pattern[37-40]. Adjusting

the values of the parameters allows the models to simulate different phyllotactic patterns seen in plants[38; 39]. Some of the properties of auxin dynamics bear similarity to those observed for Spätzle in the *Drosophila* embryo, which organizes the location of the contraction at the midline during gastrulation (see below).

Transcription factors interact with auxin similar to a polar coordinate system to determine the location of the auxin maxima around the meristem. As previously discussed, auxin maxima determine the locations of organs around the circumference of the meristem. Auxin maxima form on the boundary between concentric rings of the adaxial (leaf top; specified by HD-ZIP class III) and abaxial (leaf bottom; specified by KANADI) transcription factors (Figure 1B)[41-43]. The adaxial and abaxial transcription factors both inhibit auxin response, which results in the highest levels of auxin signaling on the boundary in between the rings. This higher auxin signaling feeds back on PIN1 orientation, causing auxin maxima to form on this boundary[41]. Together with further suppression of auxin signaling, this prevents organ primordia from forming in the center of the meristem, which maintains undifferentiated stem cells[44; 45]. Thus, the new primordium forms overlapping the boundary between *HD-ZIP* class III and *KANADI* expression domains, inheriting its adaxial abaxial identity from the meristem (Figure 1B).

In species with compound leaves, such as *Cardamine hirsuta*, the periphery of the developing leaf (known as the margin), initiates leaflets much like the initiation of organs on the meristem. The leaflets have fast growth and the cells surrounding the leaflet have suppressed growth[46; 47]. PIN1 generates auxin maxima along the leaf margin which initiate the outgrowth of leaflets and CUC (and the homeodomain protein RCO[46]) suppresses growth at the boundaries between leaflets (Figure 1C)[47-50]. Thus, differential growth of nearby regions shapes the leaf, and the degree of differential growth has been evolutionarily modified to diversify leaf shape.

*Animals: Signaling gradients establish differential contraction leading to germ layer invagination*

Epithelia are animal tissues that consist of sheets of physically linked cells that have apical-basal polarity across the sheet[51]. Epithelial tissues within developing animal embryos must deform (i.e. fold, stretch, compress, and fuse) to generate three-dimensional structures[15; 52], similar to the physically linked cells of the plant epidermis. During gastrulation, animal embryos transform from a single-layered epithelial sheet into multiple distinct germ layers[53]. Such a massive reorganization involves a variety of cell behaviors, including division, migration, rearrangement, and cell shape change. Here, we use a cell shape change, apical constriction, as a case study to examine the link between signals and three-dimensional tissue shape[54]. Apical constriction results when the apical surface of cells contract faster than their neighbors and more than on the basal side. When multiple cells constrict their apical surfaces collectively, the contractile forces are propagated throughout the epithelium, often resulting in inward bending (invagination) of the tissue sheet and internalization of the constricting cells[55].

In *Drosophila* gastrulation, morphogen and transcription factor expression patterns define the timing and location of tissue invagination, similar to the combination of auxin and transcription factors in the plant meristem. One important morphogen during *Drosophila* gastrulation is the Toll receptor ligand Spätzle, whose graded concentration on the ventral side of the embryo promotes a graded activity for the transcription factor Nuclear factor kappa B (NF-κB) or Dorsal (Figure 1D)[56-58]. The patterning of high and low contractility is critical for invagination because mutants that increase contractility in the surrounding ectoderm or create a broader distribution of contractility can disrupt mesoderm internalization and/or change tissue shape as it invaginates[59-62].

Spätzle itself is activated in a wide pattern, but is concentrated at the middle of the ventral surface (ventral midline) through a shuttling mechanism. Spätzle is produced as an inactive ligand that must be cleaved for activation[63]. After cleavage, active Spätzle rebinds and forms a complex with its N-terminal prodomain, which subsequently promotes diffusion of the complex[64]. Because the prodomain is released by binding to free Toll receptor and free Toll

receptor is highest flanking the ventral midline, there is a polarized, ventrally-directed diffusive flux of Spätzle complex that concentrates Spätzle signaling activity around the ventral midline over time[62]. The Spätzle shuttling mechanism is analogous in concept to PIN1-dependent auxin efflux because both signals are transported up their concentration gradient to create a local signaling maxima (Figure 1D). It is the precise spatial definition of these maxima and the resulting gradient that give shape to the invagination because mutants with two peaks have two furrows[65; 66].

After Spätzle-Toll signaling, activation of transcription factor expression specifies the presumptive mesoderm. High nuclear Dorsal, induces the expression of two transcription factors, Twist and Snail, which are both required for apical constriction[67; 68]. Twist and Snail promote apical constriction by inducing the expression of T48 and Folded gastrulation (Fog), which respectively encode a transmembrane protein and a ligand that activates a G-protein coupled receptor (GPCR), called Mist. GPCR signaling activates the RhoA GTPase and downstream actomyosin activation, which drives apical cell contractility (Figure 1D)[69-72]. Importantly, the timing and transcription rate of gene expression downstream of Dorsal and Twist is uneven across the mesoderm[73; 74]. Transcription of T48, Fog, and Mist starts as a narrow stripe along the ventral midline that spreads to more lateral regions of the mesoderm. The ventral-to-lateral spread of T48 and Fog/Mist expression mirrors the pattern of RhoA activation and apical myosin accumulation, consistent with the pattern of gene expression prefiguring the gradient of apical contractility[60; 75]. In the vertebrate neural tube, where morphogen gradients give rise to different neuronal identities[76], morphogens also lead to regionalized cell shapes, like apical constriction, basal expansion, and cell adhesive properties that are critical to neural fold elevation tube closure[77-80]. Therefore, the spatiotemporal patterning of morphogens and the combinatorial expression patterns of their downstream effectors regionalizes the presumptive mesoderm and leads to differential contractility between neighboring regions, which sculpts tissue shape (Figure 1E).

**Oriented growth and cell division also contribute to shape**

There are multiple strategies for creating shape. As discussed in the previous section, changes in growth rate/contraction of neighboring regions of a tissue is one strategy for creating out-of-plane deformation. Another strategy is to control the orientation of tissue growth or division. If the orientation of growth is aligned throughout a tissue, it drives elongation. If adjacent regions within tissue have conflicting alignment of growth it causes directional conflicts, driving the tissue to deform out of plane (Figure 2A)[24; 25]. Often these two strategies combine: differential rates and regulated orientations together create complex shapes during morphogenesis. Within a tissue, developmental regulators specify the growth/contraction rates and orientations that would occur if that region were allowed to deform free of mechanical constraints. However, the growth that results is modified by the mechanical constraints of interacting with neighboring tissues. Such growth conflicts resolve, often through rotation and out-of-plane bending of the tissue[24; 25].

Plant and animal tissues follow different strategies to elongate during development. Animal tissues can elongate by controlling the location of daughter cells through oriented division planes (Figure 2E)[81] or cells can rearrange to converge and extend[52]. In contrast, plant tissues elongate by controlling growth direction of the parent cell through cell wall extension. Since plant cells are “glued” together by the cell wall and inflated by turgor pressure, division itself does not change the shape of the cell lineage. Instead, divisions partition the shape of the parent cell into two cells (Figure 2B). Cell division is still crucial for plant development, just not for elongating the tissue. Elongation of a plant cell requires expansion to be greater in one direction than the other. Oriented plant cell growth is referred to as anisotropic growth (as opposed to isotropic growth, meaning equal expansion in all directions).

*Plants: Modify shape through regulating growth orientation*

Many insights into morphogenesis of plant organs have come from developing computational models that mimic morphogenesis. In general, these models require not only differences in growth rates, but also the orientation of cell growth. These models have been grounded in biology by testing whether they match the progression of normal development or mutant phenotypes. For example, models in which both growth orientation and rate are regulated by transcription factors are sufficient to recreate the complex three-dimensional shape of the snapdragon flower[82]. The ground state of the flower model is a tube of five upright petals that are fused at the base, which corresponds with the *cyc dich div* triple mutant (Figure 2C). This simple structure can be simulated through regions of the flower influencing the growth rate parallel or perpendicular to the specified growth orientation. The DIV transcription factor promotes growth at the rim (a band around the petal tube below the lobes), causing all the petals to curve downwards (Figure 2C). Then adding ventral and dorsal identity factors that further modify parallel and perpendicular growth allows the ventral and lateral petals to turn downwards and the dorsal petals to grow upward, as seen in the complex snapdragon shape of the wild-type flowers (Figure 2C)[82].

Regulation of growth direction is also used to create folds in the snapdragon petals through direction growth conflicts[25]. The ventral and two lateral petals are fused along most of their length in wild type. At the boundaries between these three petals, the tissue juts out of plane to form folds. The model of snapdragon petal development can be further modified to generate this deformation by adding conflicts in the specified growth direction (Figure 2A), which was validated biologically in petal tissues. Rebocho et al. used the *div* mutant, which has less deformation, to determine how growth conflicts in wild type create folds. In wild type, more cells are growing at angles perpendicular to each other whereas cells in *div* have more variation in growth direction. This matches the modeling in which perpendicular growth creates organ-scale deformation such as folds through directional conflict. Therefore, growth direction can create

both differences in curvature between petals and sharp folds within a tissue, depending on the angles of growth.

Models suggest that cell polarity fields orient tissue-wide alignment of growth. There is biological evidence of such tissue wide polarity fields. For example, when ectopically expressed, the protein BASL localizes to the proximal side of all epidermal cells in the leaf, suggesting these cells have a tissue wide polarity[83]. BASL and PIN1 polarities are orientated opposite each other within cells, suggesting that their polar localization reveals an organ-wide proximal-distal axis (Figure 2B)[83]. It is highly unlikely that BASL itself regulates epidermal cell growth orientation, since normally it is expressed only in stomatal lineage cells. However, the coordinated polarity of BASL in epidermal cells across the tissue is convincing evidence that such an organ wide polarity axis does exist. Polarity factors that orient growth remain to be identified.

Many developing plant organs need to elongate as they develop. For example, leaves elongate from a dome-shaped primordia into a flattened sheet. Leaf growth can be modeled by elongating regions of a tissue in a unified direction. The model starts with a hemisphere of similar shape to the organ primordia that is split into two equal halves (corresponding to the two faces of a leaf: adaxial and abaxial). Both halves are specified to grow anisotropically oriented from the base towards the top-most point of the hemisphere. This simulates cells elongating to a greater extent along the proximal-distal axis than either medial-lateral or abaxial-adaxial orientations. The model achieves a flattened sheet with two leaf faces (representing the adaxial and abaxial epidermis) (Figure 2D top)[84].

Planar leaf growth occurs due to a lack of conflict between adjacent elongating surfaces. Plant organs are composed of multiple cell layers and growth must be coordinated between these layers[85]. Conflicts in area or growth rates of the surfaces of a leaf cause out of plane curvature (Figure 2D middle and bottom)[24]. This occurs in the development of spherical carnivorous traps, which are modified leaves, in the species *Utricularia gibba.* Although early

leaf primordia and trap primordia have similar morphology, as development continues, the traps curve so that the sheet-like tissue forms a sphere instead of a plane. Trap primordia have restricted spatial expression of HD-ZIP class III identity factors for the adaxial (top) leaf face. Thus, the leaf model was modified accordingly so that the hemispherical “primordia” is not split into equal adaxial abaxial halves as it is in flat leaves, but instead into a larger abaxial region and a smaller adaxial region (Figure 2D bottom). Each region elongates, but the disproportionate initial areas cause the model curve towards the smaller adaxial side. This initially creates a cupped shape and then continues curving into a more spherical shape to match the trap (Figure 2D bottom)[84]. Trap curvature results from disproportionate initial areas whereas ovule curvature results from differential growth rates. The posterior ovule epidermis (integument) grows faster than the anterior epidermis, generating the curved shape of the ovule[86]. However, both scenarios create differences in size of adjacent regions which then causes the elongating organ to curve towards the smaller region. Thus, oriented growth works in conjunction with growth rate differences between adjacent elongating surfaces to create the variety of organ shapes within a plant and in different species.

*Animals: oriented cell division increases surface area*

Animal tissue expansion is primarily promoted by cell division. Different phases of the cell division cycle can promote expansion in different ways. First, mitotic entry in animal cells is associated with cell rounding[87-89]. This rounding in an epithelial context often is associated with an increase in the cross-sectional area of cells in the epithelial plane (Figure 2E)[90]. Second, the mitotic spindle defines the axis of cell division and the placement of the daughter cells, both of which are important for redistributing mass and regulating topological packing in the tissue[91]. Because spindle orientation can be regulated, regions of the epithelium can be remodeled by oriented cell divisions.

Cell divisions occur immediately following *Drosophila* mesoderm invagination. By expanding their cross-sectional surface area in the epithelial plane, mitotic cell rounding in the dorsal region of the embryo compensates for the majority of tissue area lost from ventral mesoderm internalization[92]. Artificially juxtaposing regions of rounding (expanding) cells with regions of constricting cells can result in ectopic invaginations[93]. An instance where mitotic rounding has been shown to promote a natural invagination is in the *Drosophila* tracheal pit, where mitotic rounding of both invaginating tracheal and surrounding non-tracheal cells accelerates invagination (Figure 2F)[94]. Overall, mitotic rounding coordinated with constriction can facilitate invagination due to differential tissue expansion/contraction in distinct regions.

Cell division orientation with respect to a tissue axis can alleviate stress induced by anisotropic growth/expansion (Figure 2G). During *Drosophila* gastrulation, oriented cell divisions are aligned with the axis of expansion[92; 95; 96]. During zebrafish gastrulation and neurulation, oriented cell divisions are also involved in axis extension[97]. In the *Drosophila* wing disc, division orientation aligns with axes of tension to dissipate stress during organ growth[98; 99]. In zebrafish epiboly, an event where embryonic cells spread over the yolk cell, oriented cell divisions prevent a buildup of anisotropic tension during embryonic tissue expansion[100]. It was shown that artificially introducing anisotropic tension into cultured epithelial monolayers, causes oriented divisions, which dissipates stress in the tissue[101].

The direction along which a cell divides and new daughter cells are formed is determined by the integration of many molecular and mechanical cues, including cell shape, that orient the mitotic spindle machinery (Figure 2H)[102; 103]. Spindle orientation is influenced by pulling forces between astral microtubules and a cortical complex of Dynein/Dynactin, NuMA, LGN, and Gαi (or other membrane anchors)[104]. The position of tricellular junctions and the presence of LGN/NuMA at these junctions is one way that cell shape influences spindle position[105; 106]. *In toto* imaging of mouse embryos revealed that orientation of cell division (either symmetric or asymmetric) is determined by competition between cell shape and apical domain

cues[107]. When these two cues are in conflict with one another, the orientation of division is directed by the stronger of the cues, suggesting a “tug-of-war” like competition between cues. This interplay between cell shape and the apical domain also facilitates proper tissue compartmentalization, further ensuring morphogenic robustness and patterning of the early mouse embryo. Altogether, such mitotic events serve as one example of how cells integrate competing cues and their influence on tissue organization.

**Local and global mechanical feedback reinforces patterns of differential growth and contraction**

Large-scale forces feedback on a wide range of cell behaviors. In this section, we will focus on how mechanical forces influence cytoskeletal alignment in animals and growth orientation in plants to reinforce growth and contractility patterns specified by signals. Mechanical feedback can also cause cells to alter their behavior, enabling unique patterns to emerge. In this sense, mechanical feedback can allow developmental events to organize themselves.

*Plants: Mechanosensitive auxin transport and cytoskeletal alignment further polarize differential growth*

Plant cells respond to mechanical stress both through polarized auxin transport, which promotes growth, and by microtubule organization, which influences cell growth direction. PIN1 localization responds to mechanical stress, which suggests mechanosensing mediates feedback between auxin transport and auxin mediated growth. For instance, increasing mechanical stress in a tissue causes increased PIN1 localization to the plasma membrane, which results in increased transport of auxin up its concentration gradient[108]. Changing the direction of tension in a tissue through cell ablation causes PIN1 in the neighboring cells to reorient its polarity (Figure 3A)[37]. This suggests that direction of auxin transport is also affected

by mechanical signals. Since PIN1 both increases and changes in localization in response to mechanical stress, this creates feedback in which auxin causes growth, which then causes more auxin to be pumped towards the growing cells[37].

Oriented growth is also reinforced through mechanical feedback mediated by microtubule arrangement. Plant cells have cortical microtubules that are not connected to centrioles and are located directly beneath the plasma membrane. These cortical microtubules dictate the orientation of cellulose microfibrils by acting as tracks for the cellulose synthase[6]. Cellulose microfibrils are long chains of sugars that are a major component of the plant cell wall (or extracellular matrix)[1; 3]. They act as strong reinforcements in the cell wall constraining growth in the direction parallel to microtubule orientation (Figure 3B)[2]. Disrupting cellulose orientation with a mutation in *cellulose synthase interactive1* alters cell growth direction[109]. Microtubules reorient in response to mechanical stress in a manner that should resist the stress by orienting subsequent cellulose deposition(Figure 3A)[110; 111]. Microtubules respond to mechanical stress generated by cell geometry or by tension. If protoplasts (single cells with the cell wall removed) are placed in wells that constrain them into a rectangular cell shape, microtubule alignment is sensitive to both tension and the curvature of the cell membrane. When the turgor pressure inside the cell is high, the microtubules align with the short axis of the cell because the shortest axis experiences more tension. If the turgor pressure is low, the microtubules align with the long axis of the cell which has less curvature, and presumably accommodates microtubule stiffness[112]. Microtubules also orient in the direction of tensile stress within a tissue (Figure 3B)[113]. Therefore, growth generates tension which orients microtubules, and then microtubules limit growth parallel to the microtubules through orienting the synthesis of cellulose, reinforcing the growth direction.

Interestingly, neither microtubules nor PIN1 rely on each other for their response to mechanical stress, rather they independently respond to mechanical stress (Figure 3A,B)[37]. However, both affect growth which could create new patterns of stress that then feed into the

dynamics of both. Microtubule response to mechanical signals is necessary to refine the morphology of organ primordia[110] initiated by auxin maxima. The boundary region adjacent to the initiating primordium has higher tensile stress due to its slow growth and the juxtaposed fast growth of a primordium, as well as the concave shape of the tissue[110]. Accordingly, the microtubules at the boundary surrounding the initiating organ become highly aligned, or anisotropic, as the organ emerges (Figure 3C)[110]. The microtubule severing mutant, *katanin,* has a dampened microtubule response to mechanical perturbations. At the boundary region, the microtubules are less aligned and the concave morphology is less distinct[111]. These results suggest that patterning of adjacent fast and slow growth creates an initial shape and mechanical stress. Then microtubules respond to the stress, reorient, and direct deposition of cellulose to reinforce the shape as it continues to grow.

Since microtubules affect growth direction, tissue-wide microtubule alignment is important for organizing directional growth, and therefore elongation. Both leaves and sepals (leaf-like organs that enclose flower buds) grow into a flattened shape which has two layers of epidermis surrounding several layers of mesophyll tissue in the center. In the growth of flattened organs like leaves and sepals, microtubule orientation in the top-bottom (abaxial-adaxial) direction restricts growth along this axis, which flattens the organ as it grows[114]. High levels of stress are predicted along the abaxial-adaxial axis, so the combined microtubule orientation and growth restriction suggests that positive mechanical feedback organizes the directional growth and causes shape change from a rounded primordia to a flattened, elongated mature organ.

In other instances, mechanical feedback sculpts the shape of the organ, specifically the sepal. During sepal development, slowing of cell growth progresses from the distal tip toward the proximal base (basipetal gradient)[115-117]. This growth pattern creates tension between regions with different growth rates, which causes microtubules to reorient and limit growth. Modeling suggests this supracellular microtubule alignment restricts the width of the sepal tip (Figure 3D). This is supported by the narrowed sepal tip in a *spiral2* mutants with a heighted

microtubule response to stress and a widened sepal tip in *katanin* mutants, which has a dampened microtubule response to stress[111; 116]. Therefore, growth triggers microtubule feedback which changes growth and affects organ shape.

Mechanical forces influence the orientation of plant cell divisions, similar to animal cells. However, in plants the division plane only affects the patterning of cell size and shape rather than affecting the shape of the tissue. In 1888, Errera proposed that plant cells divide along the shortest possible division plane that halves the cell volume, like two soap bubbles[118]. This rule has been since modified to view division planes as probabilistic based on their area[119; 120], but also affected by the supracellular stress pattern[121]. This is a tug-of war similar to animal cells– the plant cell division plane depends on whether cell shape or supracellular stress creates more tension in the cell. Before the plant cell enters mitosis, a structure formed by microtubules, called the preprophase band, marks the future division plane of plant cells[122]. Microtubules are influenced by mechanical forces, so mechanical stress also influences the division plane. Thus, cells in the meristem, which experience low tensile stress, are more likely to divide along shorter planes whereas boundary cells that are compressed between the meristem and growing organ primordia are more likely to divide along longer planes that are parallel to the direction of tensile stress (Figure 3E)[121]. Cell division orientation is particularly important for creating air spaces in mesophyll, which is a tissue that underlies the epidermis and possesses less densely packed cells. The microtubule associated protein (MAP) CLASP is necessary for alternating division plane orientation in order to create clusters of cells with air pockets in the center[123]. CLASP promotes microtubule alignment to cell-shaped derived stress rather than tissue-wide stress[124]. Thus, mechanical stress influences factors that pattern growth rate, growth orientation, and division orientation, such as PIN polarity, cortical microtubule alignment, and pre-prophase band orientation. This creates feedback between growth and mechanical signals, leading to the reinforcement and emergence of patterns and morphogenesis.

*Animals: Feedback between neighboring cells affects and reinforces 3D shape*

While pre-patterned combinations of transcriptional signals can promote certain patterns of cell shape and mechanics, these prominent genetic programs do not operate in isolation to determine a particular cellular outcome. Morphogen gradients do not only encode positional information that gives rise to the spatial patterning of transcriptional activity and expression but also regulate regionalized activation of mechanical forces that are generated and propagated throughout the tissue (Figure 3F-G). The intrinsic mechanical properties of cells and the forces they generate, are also cell signaling influencers that promote more complex patterns of cell behavior than transcription alone.

Positive feedback between the direction of tissue expansion and the direction of cell growth is also a principle in animal cells. During *Drosophila* gastrulation, mechanical forces lead to the planar cell polarization of Partner of inscuteable (Pins, LGN in mammals) and oriented cell divisions[92; 95; 96; 125]. This polarity leads to oriented cell divisions that align with the direction of tissue movement during gastrulation[92; 96; 126]. Interestingly, this principle and the role of cell polarization in directing tissue expansion during *Drosophila* gastrulation is analogous to the role of Pin-formed1 (PIN1) in positively reinforcing growth directions in plants. In both cases there is a protein that polarizes to one side of cells and promotes directional extension/growth. Some key differences are that in plant tissues this polarity directs the flow of auxin whereas in animal cells the polarity influences the direction of cell division and thus mass redistribution. Like in plant cell growth, the connection between forces and the orientation of the cytoskeleton is also critical to tissue shape. Apical constriction in mesoderm cells in the absence of opposing contractile force occurs isotropically[127; 128]. However, models based on the geometry of the contractile mesoderm tissue show that forces produced during mesoderm invagination feedback on cell shape – via mechanical competition – to make apical constriction anisotropic (Figure 3H)[75; 127; 129]. In addition, the resistance to contractile deformation aligns actomyosin fibers along the anterior-posterior axis of the embryo to promote oriented tension[130]. Recent modeling and

experiments of mesoderm invagination have also shown that oriented tension promotes furrow formation between two anchor points by straightening the tissue along its curved anterior posterior axis, which pulls (or 'knifes') the center of the mesoderm inward like the action of a cheese cutter on cheese[131]. Tension anisotropy also suppresses folding perpendicular to the anterior-posterior axis, ensuring the shape of a long narrow furrow along the ventral midline that is robust (Figure 3H)[132]. Overall, mechanical competition and the inherent shape of the embryo act as cues to orient the actomyosin cytoskeleton and tension during mesoderm invagination, which reinforces shape, similar to the role of microtubule alignment during plant organ emergence.

Mechanical interactions can reinforce spatial differences in morphogen signaling or genetic programming. Prior to *Drosophila* mesoderm invagination, cells positioned along the ventral surface of the embryo share the same genetic program, expressing both Twist and Snail (Figure 3F-G). However, F-actin levels within the lateral region are depleted in the mesoderm relative to cells in the neighboring ectoderm. As the tissue begins to invaginate, lateral F-actin accumulates in a pattern distinct from Dorsal/Twist expression or the activation of myosin at the apical surface[60; 74; 75]. While mesoderm cells at the ventral midline will apically constrict to promote inward bending of the tissue, mesoderm cells that are positioned slightly further from the ventral midline will instead expand their apical surfaces and stretch toward the furrow (Figure 3G). Such differences in cellular behavior, either apical constriction or apical stretching, can arise from the inherent mechanical differences in cells at the start and can be reinforced by dilution of the actomyosin network that results from stretching[133]. In contrast to *Drosophila* mesoderm invagination, *Drosophila* endoderm invagination is organized as a spatiotemporal wave where constriction of central endoderm cells activates contractility in neighboring cells setting up a propagating wave of apical constriction[134; 135]. Thus, mechanical competition or feedback can create situations, where initial force controls cell behavior by stretching cell apices.

**Dynamics and spatiotemporal averaging generate robustness in tissue shape**

Heterogeneity and stochasticity are observed in both growing plant tissues and contracting animal tissues. Neighboring and nearby plant cells can have different growth rates and those growth rates change in time. Similarly, apical constriction can occur stochastically across cells in a contracting tissue. This heterogeneity is also observed at subcellular resolution – between cell walls within a plant cell and fluctuating RhoA activation during apical constriction. However, these highly heterogeneous and dynamic cell growth/contraction rates average over time to give rise to highly reproducible shapes whether organs or furrows. While the purpose of heterogeneity is not yet fully understood, a combination of modeling and experimental evidence points towards heterogeneity as a strategy to effectively and robustly form shape.

*Plants: Robust organ size and shape through spatiotemporal averaging of heterogeneous growth rate*

Developing plant tissues exhibit heterogeneity in cell growth rate. Cell growth rate can have up to a six-fold difference several cells apart and neighboring cells can have up to a three-fold difference (Figure 4A)[136; 137]. This is counterintuitive considering the leaf epidermal cells are "glued" together by their cell walls and cells do not slip relative to one another. However, the difference in growth of neighboring cells is made possible by heterogeneity on a subcellular scale–different portions of the cell wall within a cell have growth rates that can differ several fold (Figure 4A)[136]. Similar to leaves, nearby cells within a sepal have different growth rates at a given time[115; 138]; however, they all reach the same maximum relative growth rate over a larger timespan[138], suggesting some constraints within the variability. In both sepals and leaves, the fast growth rates of stomatal lineage cells contribute substantially to the cell growth heterogeneity[117].

In wild-type plants, the observed cell growth heterogeneity averages over longer time intervals to produce smooth and robust organ growth. Models demonstrate that if the same cells grew fast for the entirety of organ development, the final organ shape would be irregular, with patches that overgrew compared to other parts of the tissue (Figure 4B)[115]. On the other hand, if a cell changes its growth rate over time throughout the development of the organ, modeling demonstrates that the growth rate averages out over time and space to produce a regular and reproducible shape. Thus, growth heterogeneity must average both temporally and spatially. The mutant for the mitochondrial protease *ftsh4* has variable mature sepal size and shape and correspondingly has less variation in cell growth rates[115]. Lack of variation in *ftsh4* cell growth rates produces the sepal phenotype because the growth is patchy rather than averaged. Interestingly, averaging is also present in orientation of growth (Figure 4C). Wild-type sepal cells have variable direction of growth over 24 hr intervals but have a coordinated tissue-wide growth direction over 48 hr intervals. *ftsh4* growth directions do not average over 48 hr intervals and remain patchy[115]. This mutant analysis suggests that averaging of both growth rate and orientation are necessary for reproducible sepal shape to emerge from heterogeneous cell growth.

It is unclear how the observed cell growth heterogeneity is created or its level regulated. Stochasticity in gene expression is one possible source of cell growth heterogeneity. Increasing transcriptional noise by disrupting the Pol-II machinery does increase growth heterogeneity[139]. However, this very high level of transcriptional noise in mutants disrupting Pol-II machinery is not advantageous to the plant since these mutants have softened cell walls and irregular disrupted sepal morphology[139]. Even in wild type, variation in expression level of cell wall biogenesis genes in individual sepals is associated with altering mature sepal width and curvature[140]. Thus, while wild-type levels of cell growth heterogeneity averages to promote organ shape reproducibility, excessive heterogeneity does not average during the course of development, and it is therefore deleterious to reproducible organ shape.

On an organ level scale, mechanical feedback reinforces patterns of growth rate and orientation; however, computational modeling suggests that mechanical feedback also modifies the level of growth heterogeneity at cellular scales within the tissue. One model assumes cell growth is heterogeneous from stochasticity in synthesis of the cell wall, and that growth of a cell creates mechanical stress in neighboring cells. Neighboring cells respond to stress through microtubule/cellulose re-orientation which affects growth rate and orientation. The degree to which cells respond to mechanical stress feeds back on the heterogeneity of cell growth[141]. High levels of mechanical feedback can cause cells to restrict growth in response to fast growth by their neighbors. This accentuates differences in growth between nearby cells thus increasing heterogeneity[111]. On the other hand, low levels of mechanical feedback dampen growth heterogeneity[141]. Differentiation of trichome cells, or unicellular hair cells, is a common source of mechanical stress during sepal development. Since the bases of trichome cells initially grow faster, and later slower than the surrounding cells this puts stress on the neighboring cells (Figure 4D)[117; 142]. In WT, the neighboring cells rearrange their microtubules circumferentially around the trichome to buffer the mechanical stress and prevent change in organ shape. *katanin* and *spiral2* mutants, which have a dampened and exaggerated response to stress respectively, have variation in sepal shape based on trichome number because these mutants cannot buffer the mechanical effects of the growing trichomes[142]. Thus, in different cell contexts, the wild-type plant either promotes or reduces cellular heterogeneity to achieve organ shape reproducibility.

*Animals: Dynamics and heterogeneity in uniform tissue contraction*

Cells expend energy to exhibit dynamic and heterogeneous behaviors. Cytoskeletal turnover – such as actin / microtubule assembly and disassembly - associated with nucleotide hydrolysis, creates cell dynamics. In some cases, these dynamics result in cell shape oscillations and net cell shape change that happens in a sporadic or stepwise manner[143-145].

Behaviors such as actomyosin pulses and waves cause the cells of a tissue to exhibit heterogeneous contractile activity at a given moment in time[146]. At the tissue level, cells do not all contract at the same time, but contract in a staggered and sometimes stochastic manner (Figure 4E). What is the function of this heterogeneity and is it important?

During *Drosophila* mesoderm invagination, apical constriction initiates in a manner that is initially heterogeneous or stochastic across cells of the tissue[70; 147; 148]. Mesoderm cells apically constrict when there is a 'pulse' of myosin accumulation and cell shape is stabilized between pulses (Figure 4F)[149]. Prior to this stepwise contraction, cells exhibit myosin pulses that are ineffective at constriction, with cells often relaxing between pulses[150; 151]. The mechanism that stabilizes cell shape between pulses is still poorly understood, but endocytosis, the spectrin cytoskeleton, RhoA activation, and cytoskeletal coupling to adherens junctions have all been implicated[150; 152-155]. Spatial and temporal correlation analysis in the *Drosophila* mesoderm has demonstrated that myosin pulses do not frequently co-occur, but that myosin pulses in one cell tend to follow myosin pulses in neighboring cells with a 30-60 second time lag[151]. This is also the case for other contractile *Drosophila* tissues, such as during dorsal closure, where contractile amnioserosa cells have anti-correlated constrictions[156].

Actomyosin pulsing is associated with and requires actin and myosin turnover[144; 145; 157-162]. Furthermore, research has revealed an entire spectrum of RhoA signaling behaviors, such as pulses, waves, and flashes, that exhibit a repeated cycle of autocatalytic signal activation and delayed negative feedback (Figure 4G)[160; 163-167]. This RhoA 'excitability' requires RhoA activation by Guanine nucleotide exchange factors (GEFs) and inhibition by GTPase activating proteins (GAPs), which results in a RhoA GTPase flux – cycling between GTP and GDP-bound forms[154; 160; 167-169]. When RhoA inhibition is disrupted, apical constriction initiates and is more continuous and synchronous, suggesting that pulsing is not required for constriction *a priori*. However, modulating RhoA/myosin activity in the *Drosophila* mesoderm affects tissue shape

and when knock-down of RhoA inhibitors (RhoGAP) is severe, invagination is compromised[60; 154].

Asynchronous apical constriction also occurs during neural tube closure in *Xenopus* and the zebrafish forebrain[170-172]. In *Xenopus*, apical constriction pulses are associated with cell autonomous spikes and propagating multicellular waves of cytoplasmic $Ca^{2+}$. $Ca^{2+}$ spikes are followed by apical actin remodeling (Figure 4G)[170; 172]. The propagating multicellular waves of $Ca^{2+}$ were followed by deformation of the neural plate[170]. Treatment of neurula-stage embryos with a drug that interferes with $Ca^{2+}$ reuptake and increases the baseline cytoplasmic $Ca^{2+}$ concentration disrupted the apical constriction pattern and neural tube closure[172]. One limitation of this experiment was the lack of tissue specificity to the drug's effects, such that changes to cells outside the neural plate could have contributed to the defect. However, a mechanical model of tissue-level constriction while varying pulse density showed that sparse pulses were better able to constrict the tissue[170]. Similarly, mechanical models of contraction of an actomyosin cable around a wound have shown that heterogeneous contractility more rapidly drives wound healing than homogeneous activity[173]. Heterogeneous and dynamic RhoA activation and $Ca^{2+}$ activity at a subcellular level have also been shown to repair tight junctions following breakage in Xenopus embryos (Figure 4H)[174; 175]. Overall, these data and modeling experiments suggest that heterogeneous cell contractility is important for uniform animal morphogenesis and tissue integrity and more work is needed to understand their function and spatiotemporal organization that averages contractile behavior.

## Conclusion

In both plants and animals, we have examined four key principles that underlie morphogenesis, regardless of whether shape is generated by growth or contraction. First, spatial patterning of morphogens creates regions of differential growth or contraction. Juxtaposition of differently behaving cells in a developing tissue is one of the cornerstones of

morphogenesis, generating curvature that leads to organ shape. Second, control of the orientation of cell growth and division contributes to shaping the tissue. Aligned growth in plants and division in animals causes elongation of the tissue, while conflicting orientations of growth and division causes the tissue to deform out of the plane. Third, as the tissue changes shape, it experiences new mechanical stresses, which feedback to influence the behaviors of the cells. These mechanical feedbacks can refine and enhance the patterns set up by developmental signaling processes, or they can create new patterns. Fourth, cell behavior is often heterogeneous within local regions of tissue. Dynamic changes allow heterogeneity to average in space and time, producing reproducible and robust shapes. All of these principles center around the theme that shape is an emergent property of the collective behaviors of individual cells. These four principles are not exhaustive and future research is sure to uncover additional principles for morphogenesis in plants and animals.

Plants and animals evolved multicellularity independently[176], yet both create complex three-dimensional shapes following these same four principles. The fact that evolution has independently converged on these strategies for shaping tissue suggests that these are fundamental to shape generation. Of course, considering the divergence between plants and animals, the mechanisms behind these strategies are not the same. Generally, the genes involved are different; in some cases, similar functions are carried out by non-orthologous genes and signals. Still there are many gaps in our understanding of both plant and animal morphogenesis. It will be advantageous to continue to compare across kingdoms when filling these gaps.

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**Declaration of interests**

The authors declare no competing interests.

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# Figure 1

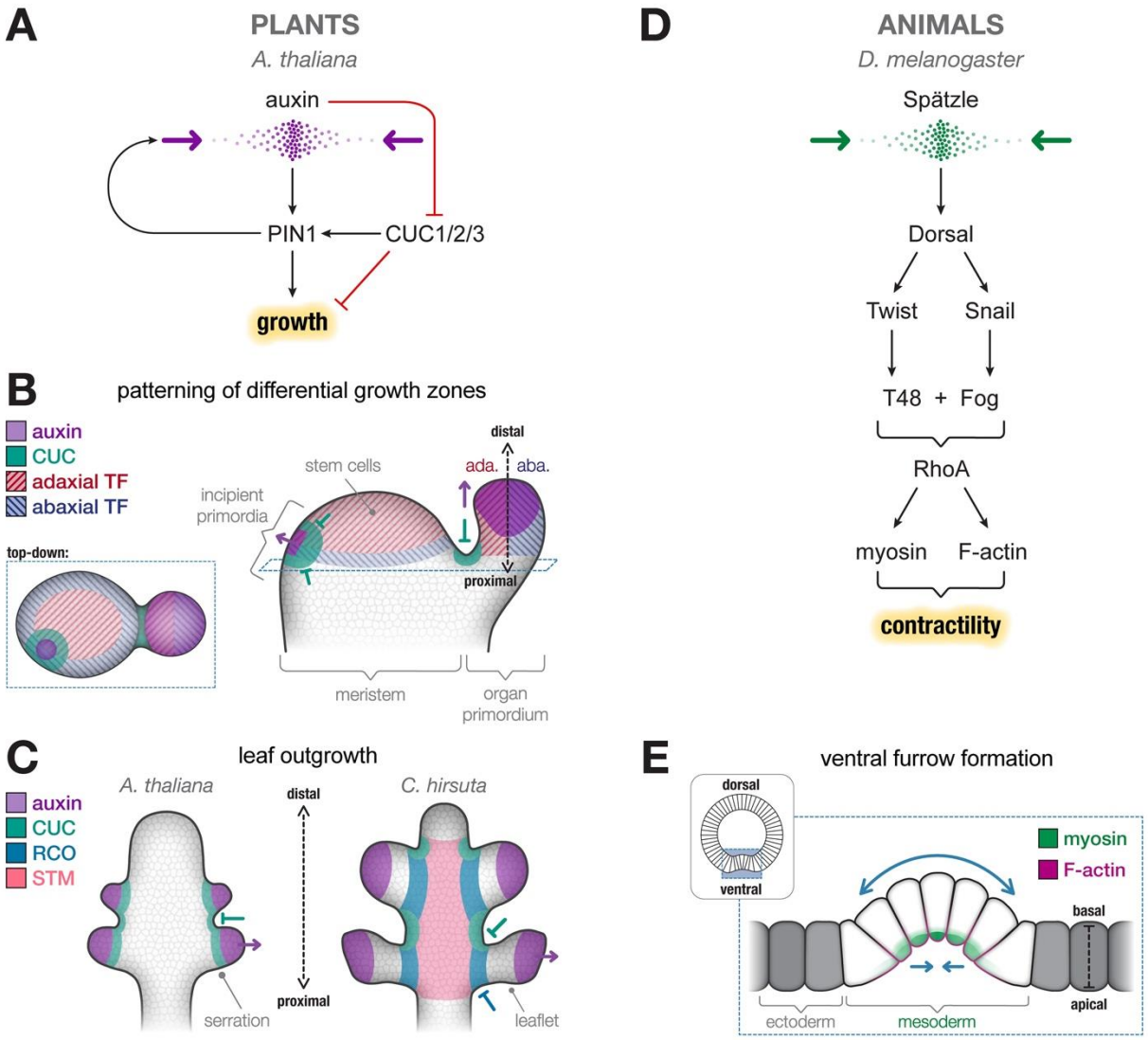


**Figure 1: Transcriptional pre-patterning. A**) Positive feedback between auxin (plant hormone) and PIN1 (auxin transporter) create auxin signaling maxima and promote growth. CUC1,2,3 repress growth. **B**) Auxin maxima (purple spots) mark the location of organ primordia and cause outgrowth of the organ. CUC1,2,3 are expressed at the boundary of the organ to accentuate the different in growth rate between the organ and surrounding cells. The boundary between adaxial HD-ZIP class III transcription factors (TF, red stripes) and abaxial KANADI transcription factors (TF, navy stripes) determine the distance of the auxin maxima from the center of the meristem. **C**) In serrated Arabidopsis leaves (left), auxin maxima promote growth

of the serration and CUC represses growth surrounding the serration. In *Cardamine* leaves (right) the growth difference is accentuated to form leaflets through the addition of STM which extends growing time and RCO which represses growth. **D**) Signaling pathway that leads to cytoskeleton activation and contractility within the *Drosophila* mesoderm. Expression and shuttling of the morphogen Spätzle (green spots) leads to high activation of Dorsal within cells along the ventral region of the embryo that will form the mesoderm. In response to high levels of Dorsal, Twist and Snail expression activates the RhoA pathway, which leads to actomyosin contractility. **E**) Schematic of the ventral side of a *Drosophila* embryo showing the invagination of the mesoderm during ventral furrow formation. Activation of F-actin (magenta) and myosin (green) promotes apical constriction of mesoderm cells (white), leading to the invagination of the tissue (blue arrows). Neighboring ectoderm cells (gray) are specified by low levels of Dorsal and therefore do not constrict.

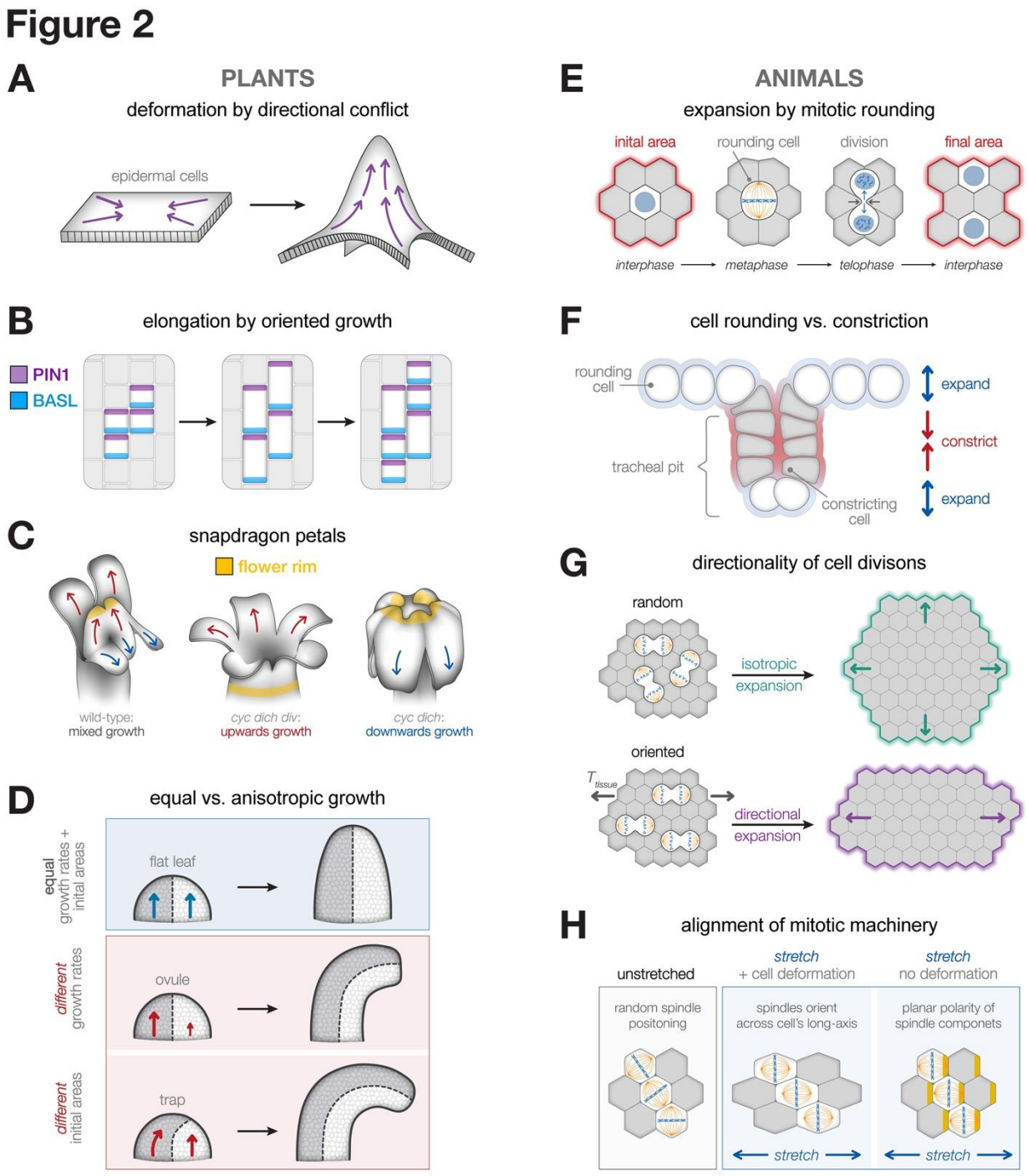


**Figure 2: Mechanisms for oriented growth: A**) A flat sheet of epidermis with cells growing in different directions causes deformation out of plane. **B**) Plant epidermal cells elongate, and then are partitioned by divisions. PIN1 (purple) localizes to the distal cell walls and BASL (blue) localizes to the proximal cell walls. **C**) Snapdragon petals of *cyc dich div* mutant grow upward

(red arrows). Adding the transcription factor DIV promotes extra growth that turns the petals downward (blue arrows). WT has identity genes that cause the dorsal petals to grow upwards and the ventral petals to turn downwards. Snapdragon petals with growth in one orientation grow upward. Snapdragon petals with growth in two directions widens the petals, which causes petals to turn downwards. **D**) Equal growth of both halves of organ (ex. flat leaf) prevents curvature (top). Unequal growth between halves of an organ (ex. ovule) causes curvature (middle). Unequal initial areas of an organ that grow in the same direction (ex. carnivorous trap) causes curvature (bottom). **E**) Mitotic rounding of dividing cells results in expansion of cross-sectional cell area. During metaphase, the dividing cell (white) will round up and push against the surrounding non-dividing cells (gray). Once telophase is completed, two new daughter cells are formed, resulting in an increase in the total surface area of the tissue (outlined in red). **F**) Competition of expanding regions (blue) vs contracting regions (red) in developing *Drosophila* tracheal pit. Mitotic rounding of cells within and around the tracheal pit facilitate the invagination of the tissue by accelerating constriction of contracting cells within the pit. **G**) Orientation of cell divisions can influence the direction of tissue expansion. Random alignment of cell divisions leads to isotropic expansion of the tissue (green arrows). In contrast, when the orientation of division is aligned along a given axis, the tissue will expand anisotropically (purple arrows) in the same direction. **H**) Physical forces can influence the alignment of the mitotic machinery and cell division. In the absence of physical cues, cell shapes and mitotic spindles can lack alignment (left). When force is applied to the tissue and cells stretch, spindles will orient along the cell's longest axis (Hertwig's rule, middle), which are aligned to due to tissue forces. In some cases, stretch can induce alignment of the mitotic machinery via planar polarity of the spindle rotation machinery, in the absence or cell shape alignment (right, yellow stripes).

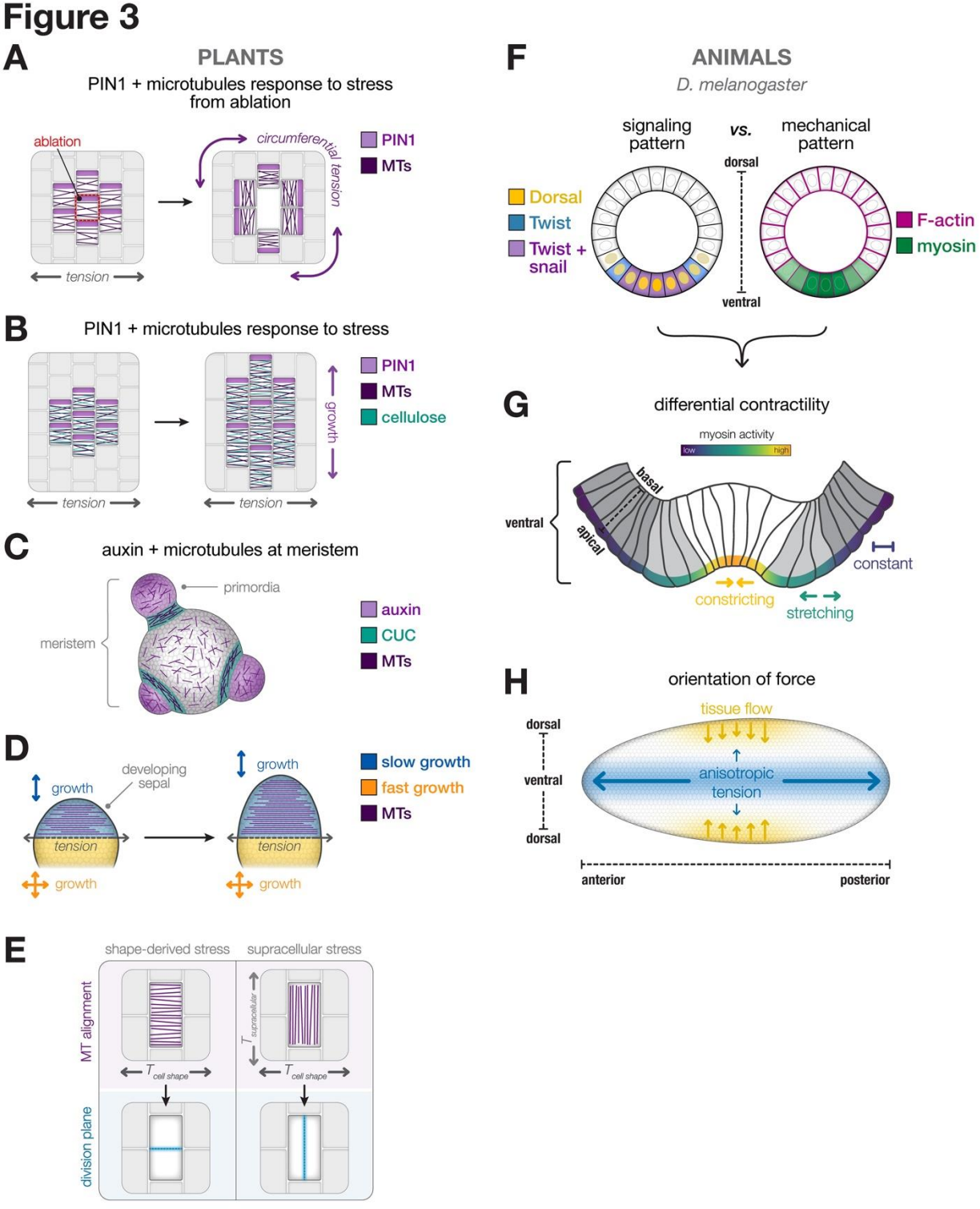


**Figure 3: Mechanical feedback. A**) Ablation of an epidermal cell causes rearrangement of PIN1 (purple) expression and cortical microtubules in response to the new pattern of stress. **B**)

Epidermal cells with medial lateral tension have PIN1 localization at the distal cell wall, cortical microtubule and cellulose (green) orientation parallel to the tension. This results in growth perpendicular to the tension. **C**) Mechanical feedback during organ initiation in which microtubules become anisotropic in the boundary region between the meristem and initiating organs. **D**) Fast growth in sepal morphogenesis recedes from distal to proximal and causes tension between the fast growing (yellow) and slow growing regions (blue). Cortical microtubules align parallel to the tension and inhibit widening of the sepal tip. **E**) Tension from cell shape promotes cortical microtubule orientation and division plane along the shortest axis. Tension from surrounding tissue promotes cortical microtubule orientation and division plane parallel to the supracellular tension. **F**) Differences between the patterning of morphogen signaling and cytoskeleton along the dorsal-ventral axis of a *Drosophila* embryo. **G**) Mechanics of mesoderm invagination, highlighting difference in levels of contractility. Mesoderm cells, which have the highest level of actomyosin activity, constrict (yellow arrows). Neighboring marginal mesoderm cells (gray) have lower levels of actomyosin activity and are able to stretch (green) in response to apically constricting cells. Ectoderm cells, which have little myosin activity but high F-actin levels, resist constricting forces and maintain their shape (purple). **H**). Orientation of anisotropic tension during gastrulation depends on embryo shape. Blue arrows denote direction of tension generated across the anterior-posterior axis on the ventral side of the embryo. Yellow arrows denote tissue flow, showing inward movement of the tissue towards the ventral surface.

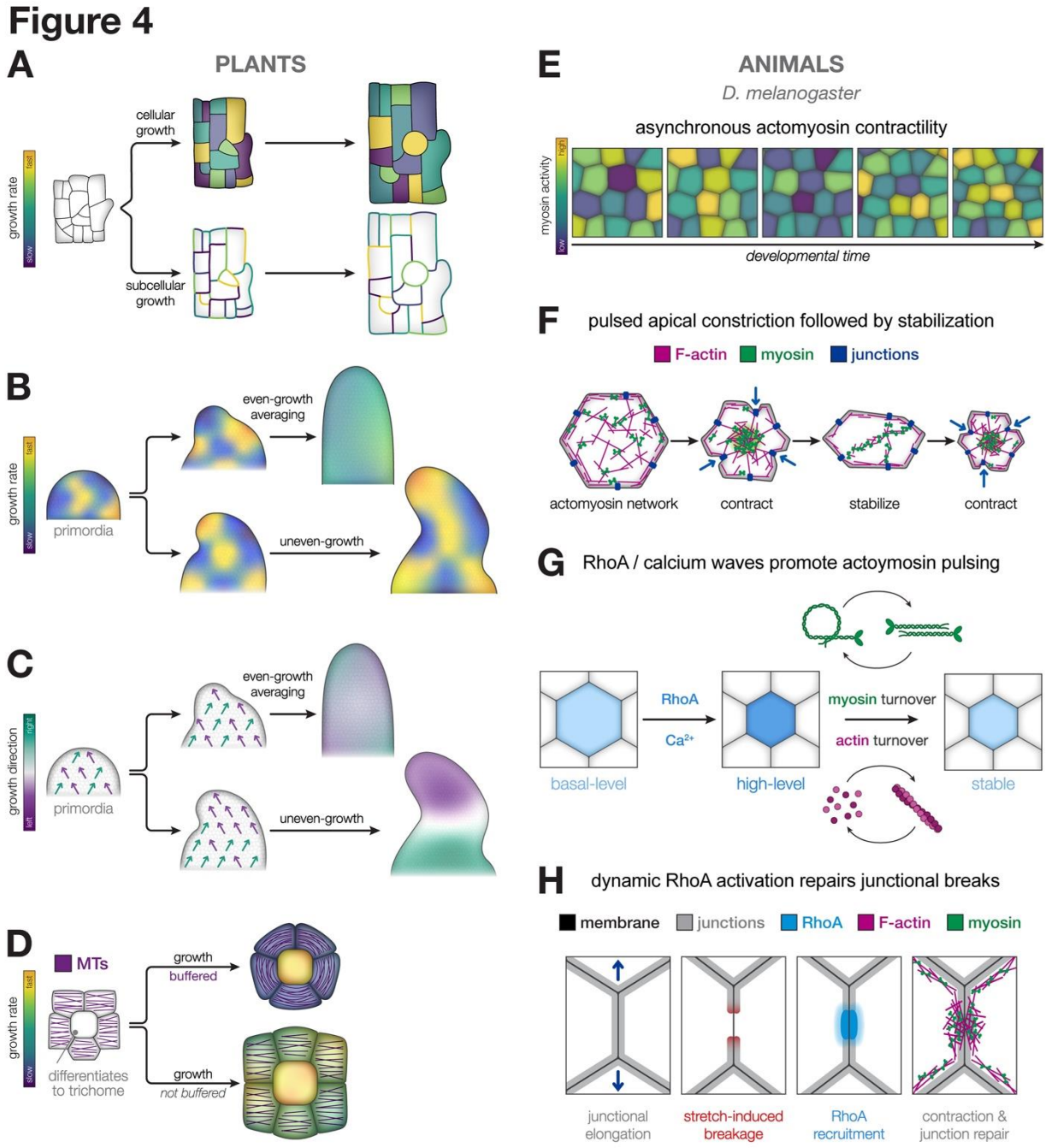


**Figure 4: Heterogeneity and collective cell behavior. A**) Epidermal cell growth rates (top) are heterogeneous within a tissue and epidermal cell wall growth rates are heterogenous within a cell and within a tissue. Growth rates are displayed as a heat map with fast growth in yellow and slow growth in purple. **B**) An organ with dynamic heterogenous growth rates that average

spatially and temporally creates even, reproducible growth (top) vs an organ with static heterogenous growth rates that do not average spatially and temporally creates uneven, variable growth (bottom). **C**) An organ with dynamic heterogenous growth orientations that average spatially and temporally creates even, reproducible growth (top) vs an organ with static heterogenous growth orientations that do not average spatially and temporally creates uneven, variable growth (bottom). Growth direction is displayed as a heat map with left as purple and right as teal. **D**) Trichome cell in the center grows fast which creates mechanical stress. Cortical microtubule response to mechanical stress in surrounding cells slows their growth, creating heterogenous growth rates (top). If cortical microtubules do not respond to mechanical stress there is less heterogeneity in growth rates (bottom), but more influence on organ shape. **E**) Actomyosin contractility is heterogenous through the tissue. Apical constriction is staggered across cells, where some cells exhibit higher levels of myosin activity compared to their neighbors. **F**) Actomyosin contractility exhibits a pulsatile behavior. Myosin motors (green) pull on actin filaments (magenta) that are coupled to cellular junctions (dark blue) and constrict the apical surface of the cell. Constriction is followed by a period of stabilization, there the actomyosin network and cell shape is reinforced. **G**) Pulses of RhoA or $Ca^{2+}$ can promote actomyosin pulsing and turnover. Cycling levels of RhoA or $Ca^{2+}$ facilitates myosin turnover (activation/deactivation) or actin turnover (polymerization/depolymerization), respectively. In the *Drosophila* mesoderm, bursts of myosin activity are followed by periods of stabilization. **H**) Dynamic RhoA activation repairs breakages in cell junctions. When breaks occur in the junctional network, a burst of RhoA activity (blue) near the breakage site recruits F-actin (magenta) and myosin (green) to promote reinforcement and repair.