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Commentary

Resolving Conflicts: Modeling Genetic Control of Plant Morphogenesis

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Computational modeling of tissue morphogenesis reveals how spatiotemporal patterns of gene activity control tissue shape by introducing several types of tissue conflict. These conflicts reflect genetic modulation of processes that influence the cellular mechanical properties and may underlie the enormous diversity of forms that have evolved in plants and animals.

In 1945, at age 64, Pablo Picasso did 11 drawings of a bull in various styles, from realistic versions shaded in tones of gray, to more abstract forms with blocks of black or white, to a highly simplified drawing comprising just a few lines (see http://www.artyfactory.com/art_appreciation/animals_in_art/pablo_picasso.htm). Which of these drawings represents the greater challenge? The more realistic or complex drawings are difficult because of the many features they contain. The highly simplified drawings are challenging for the opposite reason: deciding what to leave out.

We encounter a similar issue when applying computational modeling to development. There is the challenge of producing realistic and comprehensive models, with all the parameters and complexities we can incorporate. Or we may strive for stripped-down models that leave out many elements yet capture the essence of the process. These approaches are not disconnected. We may start with a simplified model and find ourselves adding more and more elements to account for greater details. Conversely, having built a complex model, we may explore what can be removed without changing its essential character, to arrive at a deeper understanding. It is often by moving between different levels of analysis that progress is made.

Here we discuss some of the key principles that have emerged using different levels of abstraction to model plant morphogenesis. We focus in particular on how this approach has helped address one of the great challenges in developmental biology: connecting patterns of gene expression with the generation of shape and form.

We begin by abstracting away from cells to ask what is needed for a continuous growing material to shape itself into a plant structure. Once this has been clarified, we consider how cellular properties may be incorporated into models to account for tissue-level behaviors. This is analogous to the way in which the laws of thermodynamics were established at the macroscopic level first, and then underlying explanations were found at the molecular or quantum level.

For growing tissue, the extent to which a region grows may depend not only on its intrinsic growth properties but also on mechanical constraints from neighboring regions. To clarify these contributions, we distinguish between two types of growth: specified and resultant (Kennaway et al., 2011). Specified growth is how a region of tissue would grow if it was free from the mechanical constraints of its neighboring regions. Resultant growth is how a region grows in the context of neighboring mechanical constraints, and by definition includes stretches, compressions, rotations, and curvatures that emerge from such constraints. Specified growth therefore refers to the intrinsic or active growth properties of a region, while resultant growth also includes the passive changes that arise through connectivity with other regions.

To illustrate the relationship between these two aspects of growth, consider a square sheet of tissue that grows in area but not in thickness and is marked with circular spots. Suppose each tissue region has a specified growth rate that is equal in all directions in the plane (isotropic-specified growth). If a growth-promoting transcription factor, GTF, is expressed uniformly throughout the tissue, the tissue simply gets larger (Figure 1A). Here all regions grow in a similar way without constraining each other, so resultant growth is the same as specified growth.

If, instead of being uniform, GTF expression increases linearly from left to right, nearby regions are no longer specified to grow at the same areal rate. This conflict in specified growth rates leads to the square curving into a fan (Figure 1B). Resultant growth is no longer the same as specified growth because regions have rotated relative to each other as a result of their connectivity. The curvature resolves the conflict, giving what is termed a conformal map, where angles are preserved locally (Mitchison, 2016).

If GTF is only expressed in the right half of the tissue, we obtain a skirt-like shape (Figure 1C). The initial circles have become elliptical near the middle boundary, being extended vertically on the left side and horizontally on the right side. This resultant growth anisotropy arises because conflicts in growth of the left and right halves are only partially resolved, generating residual stresses. The left regions become passively stretched vertically by the faster-growing tissue on the right, while the right regions are passively restrained from growing vertically by the slower-growing tissue on the left. Here specified growth is isotropic, while resultant growth exhibits anisotropy and rotations due to mechanical constraints. In both of the above examples, the tissue shape change and curvature are driven by conflicts between areas in the plane of the tissue specified to grow at different rates, and we refer to this situation as *areal conflict*.

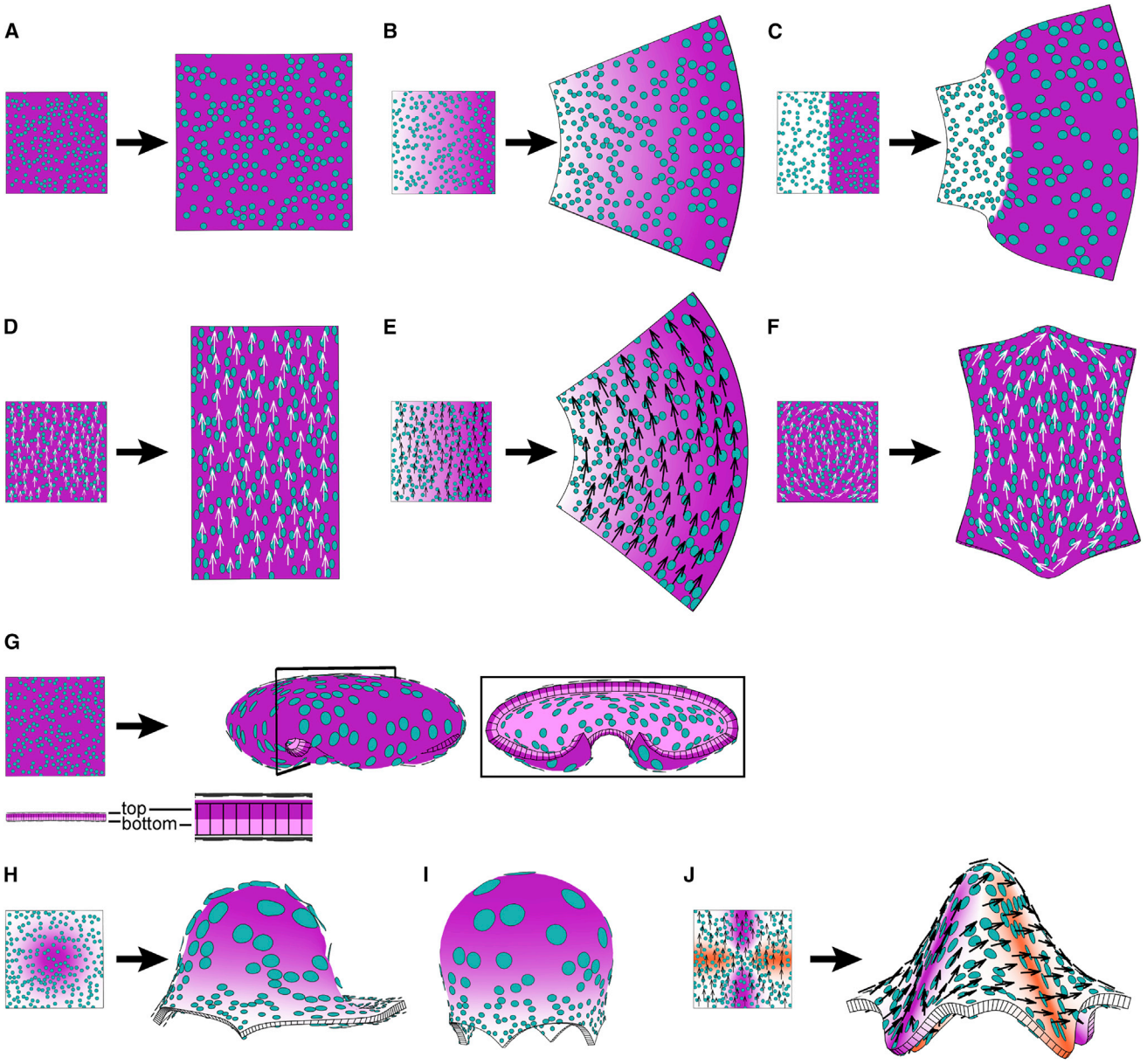

Figure 1. Generation of Deformations through Tissue Conflicts

(A) Uniform isotropic growth controlled by a growth-promoting transcription factor (GTF, purple shading) resulting in a bigger square with larger isodiametric clones.

(B) GTF expression increases linearly from left to right, promoting isotropic growth, resulting in a fan-like shape with bigger clones on the right side.

(C) GTF is only expressed on the right half of the canvas, where it promotes isotropic growth resulting in a skirt-like shape with clones near the midline boundary horizontally elongated on the right side and vertically elongated on the left side.

(D) Uniform anisotropic growth, with a proximodistal polarity field (arrows). GTF promotes higher growth parallel than perpendicular to the polarity. The square elongates to form a rectangle with vertically elongated clones.

(E) GTF expression increases linearly from left to right, promoting higher growth parallel than perpendicular to the polarity, resulting in a curved fan with elliptical clones on the right.

(F) Uniform anisotropic growth, as in (D), in the context of a curved polarity field. The resulting shape protrudes at the poles of the field.

(G) GTF promotes specified uniform isotropic growth and is expressed at a higher level in the top surface. The shape deforms into a cushion-like dome, with downward curled edge.

(H and I) GTF promotes specified isotropic growth and is expressed at a higher level in the center. When the initial tissue is flat except for small random perturbations, buckling can be upward and/or downward. When the initial tissue is slightly curved, buckling is biased and leads to a rounded dome (I).

(J) One transcription factor promotes growth parallel to the polarity (purple) while a second promotes growth perpendicular to the polarity (orange). An elongated dome is generated, with clones elongated parallel to the polarity in purple regions and perpendicular to the polarity in orange regions.

It is also possible for specified growth to be anisotropic. In this case, regions have the intrinsic property of growing preferentially in one orientation, even when mechanically isolated from neighbors. How might such local orientations be specified? One hypothesis is that they derive from residual stresses generated by differential isotropic-specified growth, such as the stresses causing resultant anisotropy near the middle boundary in Figure 1C. It has been proposed that such stresses might feed back to reinforce tissue regions in the direction of the local stress, giving specified anisotropic growth properties (Hervieux et al., 2016). However, simulations show that using local stresses to orient specified anisotropy in this manner does not allow a coherent pattern of orientations to be specified (Hervieux et al., 2016). In being guided by the pattern of residual stresses, growth feeds back to modify the stresses and destabilizes their orientations. To circumvent this problem, Hervieux et al. (2016) proposed that orientations are specified by average stress orientation across the tissue. It is unclear, however, how a sensing mechanism might perform such averaging, or distinguish between local and average stresses, both of which have the same physical character.

An alternative hypothesis is that polarity provides the orientation information that guides specified anisotropic growth. In a continuous material, polarity can be most readily implemented by taking the local gradient of a diffusible factor. If production of this factor is promoted along the bottom of our square tissue (source) and removed along the top (sink), the gradient at each position gives a vertical polarity field. The source and sink regions are referred to as *organizers of polarity*, with polarity pointing away from the plus organizer (source) and toward the minus organizer (sink). Two different growth rates may be specified for each position: a rate parallel to the local polarity and a rate perpendicular to the local polarity. If GTF increases specified growth parallel to the polarity and is uniformly expressed, the square elongates into a rectangle (Figure 1D). Each region is specified to grow in a similar manner, and resultant growth is the same as specified growth. By contrast, if GTF increases from left to right, we obtain a fan, with elliptical clones toward the right (Figure 1E). As with differential isotropic-specified growth, curvature arises because of the conflict between nearby regions specified to grow at different rates, although here the specified growth is anisotropic.

Using polarity to guide specified anisotropic growth leads to the possibility of another type of conflict. Suppose the source and sink of the diffusible factor are restricted to small regions at the top and bottom center, giving a curved polarity field with organizers at the poles (Figure 1F). With uniform expression of GTF, and specified growth rates higher parallel than perpendicular to polarity, the square curves to form points where polarity converges or diverges (sites of the organizers). Here, tissue curvature arises because of a conflict between nearby regions trying to grow in different orientations, as the polarity field is curved. We refer to this situation as *directional conflict*.

In the above examples, conflicts are largely resolved by rotations or curvature in the plane of the tissue. In other cases, conflicts may lead to out-of-plane rotations. For example, if GTF is expressed only in the top surface of our sheet and promotes isotropic-specified growth, the conflict is resolved by the tissue curving downward (Figure 1G). We refer to this situation as *surface conflict*.

Out-of-plane curvature may also arise through buckling. If GTF promotes isotropic-specified growth and is expressed at a high level within the center of the tissue, areal conflict can lead to formation of a wave or dome (Figure 1H). If the initial sheet is flat, with small random local perturbations, the direction of buckling is random, whereas if the sheet has a slight curve, generated by a small degree of surface conflict, the buckling direction and shape is biased according to the initial curvature (Figure 1I). Examples of buckling arising through areal conflict have been previously analyzed, for example in lily petals (Liang and Mahadevan, 2011).

A further type of buckling can arise through directional conflict. Suppose we have an orthogonal expression pattern of transcription factors (Figure 1J), with one factor promoting growth parallel to polarity (purple vertical domain) while the other promotes growth perpendicular to polarity (orange horizontal domain). In the context of a uniform vertical polarity field, the conflict in orientations of specified growth leads to out-of-plane buckling and formation of an elongated dome. Evidence that genetically controlled directional conflicts play an important role in 3D morphogenesis has come from the analysis of *Antirrhinum* flower development (Green et al., 2010).

These examples of tissue-level modeling show that genes may influence morphogenesis by regulating the spatiotemporal expression of factors like GTF that control specified growth rates, or by modulating polarity organizers or stress-sensing mechanisms that orient specified anisotropic growth. How might these activities be implemented at the cellular level?

A growing plant tissue can be considered as a deforming mesh of cell walls that yields continuously to cellular turgor pressure (Cosgrove, 2016). This continuous process of mesh deformation is coordinated with the introduction of new walls through cell division, keeping cell sizes within certain bounds and allowing mesh strength to be maintained as it grows.

According to this view, specified growth depends on how genes control the degree of wall extensibility and cell turgor (Cosgrove, 2016). Turgor pressure acts isotropically, while cell wall extensibility can be anisotropic because of the orientation and crosslinking of wall fibers such as cellulose. If a sheet of cellular tissue has uniform turgor and its walls have isotropic mechanical properties and yield to the pressure, the tissue gets uniformly larger. This situation corresponds to uniform isotropic-specified growth. Conflicts arise through spatial variation in turgor and/or wall extensibility. For example, the areal conflict illustrated in Figure 1H could arise if walls in the central region of a square tissue yield more readily in the plane to turgor pressure. Similarly, the surface conflict in Figure 1G could reflect walls in the upper layers of the tissue yielding more readily than those toward the bottom.

Specified anisotropic growth depends on orientation and/or crosslinking of cellulose fibers. Cellulose synthesis is guided by microtubules that exhibit dynamic patterns of alignment and realignment (Ehrhardt and Shaw, 2006). Computational

modeling of microtubule dynamics has shown that microtubule alignment can be self-organizing. There are two basic hypotheses for how such self-organization may arise (Eren et al., 2012). One is that alignment is driven by the process of zippering (bundling), whereby the plus end of a microtubule is redirected to join the path of another following an encounter. The principle is similar to that used in swarming algorithms, where ants follow the trails left by other ants. The other hypothesis is that alignments arise through differential survival, as microtubules at divergent angles are more likely to undergo catastrophes. In either case, the mechanisms would lead to each cell forming alignments irrespective of its neighbors.

For microtubule alignments to be coordinated in a tissue, their orientation needs to be biased through polarity and/or stress fields. A possible cellular basis for establishing polarity fields is through the asymmetric cellular localization of PIN auxin transporters. Various computational models have been proposed to account for the coordinated localization of PINs across tissues. These models can be classified into two types according to the patterns they generate in the context of an initially uniform auxin distribution (Abley et al., 2016). One class of model generates sites of polarity convergence and assumes that PINs become localized toward neighboring cells with higher levels of auxin. Another class of model generates head-to-tail tandem alignments. These models assume that PINs localize to membranes of high auxin efflux or low extracellular auxin. Both classes of model can generate a range of tissue cell polarity patterns by introducing auxin sources, introducing sinks (plus and minus organizers), and controlling plasticity in polarity.

Whatever model underlies the generation of coordinated polarity patterns, these patterns could influence growth orientations by biasing alignments of microtubules or other processes influencing cell wall anisotropy and loosening. The result would be different wall stiffnesses parallel or perpendicular to the polarity, giving different rates of specified growth. Directional conflicts would then arise through variation in tissue cell polarity directions and/or variation in how cells modify wall extensibility in response to polarity. Stresses may also bias microtubule alignments by affecting microtubule stability or by mechanosensation (Landrein and Hamant, 2013), or strains may alter wall properties by the creation of available space in the wall (Green et al., 2010). The relative contribution of polarity, stress, or strain on specified growth anisotropy remains to be established.

The above examples involve planar growth of a sheet of tissue. Similar considerations apply to volumetric growth. A plant primordium is a solid outgrowth, with innermost tissue typically growing in an orientation perpendicular to the surface. In lateral root primordia the conflict between interior and epidermal growth leads to the internal tissue breaking through the epidermis, while for a leaf primordium the epidermis grows and folds around the internal tissue. Isotropic-specified growth patterns underlying leaf primordium formation have been modeled volumetrically (Boudon et al., 2015). However, specified anisotropy may also be involved. In leaf primordia, PIN polarity becomes reorganized to form a convergence site in the epidermis and a connected strand of inwardly oriented PINs in the internal tissue (Bayer et al., 2009). These polarity changes may be involved in reorienting specified anisotropic growth.

Alternatively, specified anisotropy may be guided by stresses. The shoot apex has been modeled as a pressurized continuous cylinder with a yielding epidermal sheet. This geometry generates circumferential stresses, and these have been proposed to create specified anisotropy through a mechanical feedback mechanism (Hamant et al., 2008). The PIN polarity changes may then be viewed as an outcome of reading stress gradients (Heisler et al., 2010). Testing and integrating these different perspectives on polarity, stress, and anisotropy for volumetric growth will be a future challenge.

Our discussion here has focused on plants, yet many of the same principles and approaches may also be relevant to animal morphogenesis. Compared to plants, animal cells exhibit distinctive behaviors such as rearrangements, migration, and contraction, yet at the tissue level they may lead to similar morphogenetic processes. The principles of tissue conflict apply whether a region of tissue is growing or contracting. The process of convergent extension, for example, corresponds to negative growth in one orientation and positive growth in the orthogonal orientation. As with plants, the shaping and specified anisotropies of animal cells depends on cytoskeletal components, such as microtubules and actin filaments. Moreover, tissue cell polarity operates in animals in a comparable way to plants, except that the absence of cell walls allows for more direct interactions mediated by bridging proteins (Abley et al., 2013). Thus, many of the principles described here may be broadly applicable to morphogenesis in plants and animals.

Computational modeling of morphogenesis has been applied at different levels: cytoskeleton, cellular, and tissue. At each level it has allowed underlying principles to be clarified: self-organizing microtubules, mechanisms for cell polarity coordination, and the role of tissue conflicts and mechanics. Genes modulate these processes by changing the properties of the interacting components. At the tissue level, genetically controlled changes in patterns of tissue conflict may underlie the enormous diversity of shapes and forms that have evolved. At the cellular or subcellular level, these same evolutionary changes reflect genetic modulation in spatiotemporal patterns of cell properties. These studies illustrate how there is no single level of abstraction that captures everything. Rather, it is by seeing how the different levels operate and connect that modeling is helping us to arrive at a deeper understanding of the principles of morphogenesis.

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