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Quantitative approaches to uncover physical mechanisms of tissue morphogenesis

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Abstract

Morphogenesis, the creation of tissue and organ architecture, is a series of complex and dynamic processes driven by genetic programs, microenvironmental cues, and intercellular interactions. Elucidating the physical mechanisms that generate tissue form is key to understanding development, disease, and the strategies needed for regenerative therapies. Advancements in imaging technologies, genetic recombination techniques, laser ablation, and microfabricated tissue models have enabled quantitative descriptions of the cellular motions and tissue deformations and stresses with unprecedented temporal and spatial resolution. Using these data synergistically with increasingly more sophisticated physical, mathematical, and computational models will unveil the physical mechanisms that drive morphogenesis.

Keywords

morphodynamics; mechanotransduction; mechanical stress; traction force microscopy

Introduction

The physical microenvironment plays an important role in cell homeostasis, phenotype, and disease. Tissue morphogenesis, the formation of tissue architecture, is driven by genetic programs, microenvironmental cues, and the inter- and intracellular communication that connect them. As such, it is critical to understand how the physical microenvironment shapes and remodels growing tissues, and how dysregulation of the mechanical microenvironment drives disease.

Significant technological advances – both experimental and computational – have been made in recent years that enable investigations into the microenvironmental factors that influence tissue behavior. The application of techniques and analysis frameworks from the physical sciences to biological problems has revealed answers to the complex and fundamental questions surrounding the creation and maintenance of tissue form and function central to developmental, cell, and cancer biology. Here, with an eye toward understanding tissue and organ development, we review approaches to quantify the evolution of tissue

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architecture over time (kinematics) and strategies to define the physical forces that drive these morphogenetic movements (dynamics). We also discuss the powerful benefits of marrying computational models with experimental observations to investigate the physical mechanisms of morphogenesis.

The evolving tissue architecture: a kinematic understanding

The architecture of a tissue is linked to its function [1–3]. As evidenced *in vivo* [4–7] and in two-dimensional (2D) and three-dimensional (3D) engineered culture models [8–12], tissue geometry sculpts patterns of soluble factors and mechanical forces that in turn feed back to generate spatial patterns of cell behavior and further drive tissue form and remodeling. To begin to understand the complex and non-intuitive behaviors that emerge in multicellular systems, a fundamental question must be answered: how do the cells within these tissues move over time? Our understanding of the deformations that occur during morphogenesis is tightly coupled to advances in imaging and image processing technologies. Techniques for measuring cell movements and tissue deformations are simple in concept – tracking a series of fiducial markers in time and space – but complicated in practice [13,14].

In some studies, microspheres are attached to the tissue surface [15], but in many instances the cells themselves can serve as fiducial markers (Figure 1A). Cells have been fluorescently tagged with membrane dyes or transfected to label the nuclei or cytoplasm with markers such as green fluorescent protein (GFP). A non-uniform labeling distribution, necessary for accurate marker identification and tracking, can be achieved with clever methodologies, such as sprinkling metal particles coated with membrane dyes that are subsequently removed with a magnet [16] or by ensuring low transfection efficiencies. Additionally, fluorescent reporter strategies [17] or the creation of chimeric embryos [18] (Figure 1B) can be used to label subpopulations of cells with tissue-specific promoters, thereby creating mosaic tissues in which individual cells can be tracked over time. For example, the Brainbow technology uses a Cre/lox recombination system to express up to 90 discernable colors within a mosaic tissue suitable for tracking large populations of individual cells simultaneously [19–21] (Figure 1C). Similarly, RGB-marking technology uses lentiviral gene ontology (LeGO) vectors to express red, green, and blue fluorescent proteins stochastically in a population of cells [22]. The development of these genetic constructs, coupled with new techniques to design photo-switchable fluorophores that shift emission wavelengths when activated [23], permit the precise labeling of large populations of cells in 2D and 3D culture, whole organ explants, and *in vivo*.

Improvements in imaging techniques, together with the application of artificial intelligence in imaging processing, have facilitated the tracking of these cell and tissue markers in developing organs. Integrating multiphoton microscopy with intravital imaging has allowed non-invasive fluorescence imaging at greater tissue depths (up to 300 μm) *in vivo* with lower photobleaching and phototoxicity [24,25]. Frequently, *ex vivo* and culture models are imaged via confocal microscopy to track the position of the fluorophores in 3D over time. Advancements in confocal microscopy, including line scan and laser-sheet confocal, have enabled larger scanning areas with higher scanning frequencies, greater resolution, and a decreased phototoxicity so that long-term repeated imaging of live samples is possible [26,27]. Such approaches have been used to image the morphogenetic movements of growing plant roots [28], tracheal development in *Drosophila melanogaster* [29], and cardiogenesis in the zebrafish [30,31]. Finally, optical projection tomography (OPT) [32,33] and optical coherence tomography (OCT) [15,16,26,27], which use the projection images taken around a sample or optical backscattering of light through a sample, respectively, have gained wider use in mapping tissue architectures in real time as they have sufficient imaging speeds and do not require exogenous tissue markers (Figure 1D).

Using experimental techniques to label tissue surfaces and track cellular motions provides information at multiple length scales *in vivo* and in culture. At the multicellular level, tracking individual cells exposes fundamental cell shape changes and rearrangements that lead to epiboly and convergent extension [34–36] (Figure 1E), collective cell migration [37], biases in division angle orientations [38], and self-assembling cell sorting [17,18,39,40] within the tissues of interest. At larger length scales, the tissue can be approximated as a continuum and the position of markers used to reconstruct the tissue geometry at a given time point. These 3D reconstructions are then used to visualize, measure, and interact with complex geometries [35,41–43] (Figure 1D, F) or to generate anatomically accurate geometries for numerical analysis [44]. Furthermore, the 3D deformation gradient tensor can be calculated from the marker positions as they move over time, enabling the creation of deformation maps that describe the morphogenetic movements of growing and remodeling tissues and organs [15,45]. The quantitative descriptions of the cellular motions and tissue deformations that these techniques provide are critical to understand evolving tissue architectures and serve as the foundation for biomechanical analysis and the development of computational and mathematical models of morphogenesis.

The dynamics of morphogenesis

Rigorously determining the forces that drive morphogenesis is non-trivial due to the complex geometries, highly nonlinear heterogeneous extracellular matrix, dynamic cellular environment, and, frequently, the experimental inaccessibility of the tissues of interest. In addition to these technical challenges in understanding the mechanical environment, the production of forces by cells is modulated in space and time by alterations in gene expression in response to microenvironmental cues [46]. Given the complexity of the mechanical environment, the most common approach to identify the physical mechanisms that drive morphogenesis is to disrupt the system with physical (e.g. laser ablation), pharmacological (e.g. cellular contractility inhibitors and enhancers), or genetic (e.g. gene knockdown/knockout) perturbations and observe the motion of subcellular structures or tissue markers to determine the forces qualitatively. For example, the displacement of myosin II and the shape of epithelial tears following laser ablation of cellular actomyosin networks were used to measure the relative tensile forces and directions that drive anisotropic apical constriction during ventral furrow formation in the *Drosophila* embryo [47] (Figure 2A). Similarly, the deformed shape of a series of holes punched through an early stage chicken embryo can provide insight into the loading state of the tissue by identifying regions of tensile and compressive stresses and the degree of anisotropy of the stress field [16]. As a result of the complex mechanical processes involved in morphogenesis, most of the advances in our understanding of morphodynamics are from well-characterized invertebrate models. Established genetic and molecular tools and libraries of mutants, often coupled with transparent embryos, permit the investigation of forces that drive morphogenesis and the roles of various molecular pathways in that process. However, even with the multitude of techniques to determine the mechanical properties of embryonic tissues [48–52], the lack of well-defined constitutive models and our incomplete understanding of cellular mechanotransduction pathways often necessitate the use of culture models to provide more quantitative measurements of cellular forces.

Several culture models have been used to calculate cellular forces during morphogenesis. In 2D sheets of cells, traction forces are commonly calculated from the displacements of fluorescent beads [53] or the deflections of elastomeric posts [8] on the underlying substratum. Recently, monolayer stress microscopy (MSM) was developed whereby the traction stresses at the cell-substratum interface are combined with a straightforward force balance to calculate the forces at any point within the tissue [54,55] (Figure 2B). Novel microfabrication techniques have provided the ability to control the position of cells relative

to each other [56,57] and to construct tissues with defined geometries [57,58]. Micromolded 3D tissues with controlled geometries [10,59] have been integrated with 3D traction force microscopy to measure surface traction stresses on epithelial tubules and dissect the relative roles for chemical and mechanical signaling in branching morphogenesis [60] (Figure 2C–E). In addition, larger scale elastomeric posts have been used to measure the contractile properties of 3D engineered microtissues [61–63]. These culture models provide a less complex, homogenous mechanical microenvironment with well-characterized material properties that permit the study of mechanotransductive signals and the forces that drive morphogenetic movements.

Computational modeling of morphodynamics

Morphogenesis is an elaborate process that relies upon complex cellular interactions across multiple length scales. Computational and mathematical models provide a quantitative framework to describe the kinematics of the system and understand the underlying physical mechanisms that drive the cellular and tissue behaviors observed experimentally [64,65] (Figure 3A). Several types of models, ranging from simple physical models [66] to more sophisticated computational models that explicitly integrate chemical and mechanical feedback [67,68], have been used to describe these observed behaviors. Agent-based models including vertex and cellular Potts models have particular utility in describing and predicting cellular rearrangements, shape changes, and intercellular interactions [69]. For example, vertex models combined with timelapse imaging were used to show that cells undergo both apical constriction and apicobasal shortening during ascidian endoderm invagination [70]. On the other hand, models that approximate the tissues as a continuum often rely on the finite element method (FEM) to understand the evolution of tissue shape, and often excel at exploring and capturing large-scale tissue deformations [15,16] (Figure 3B–D). The main goal of all of these models is to make sense of complex cell and tissue movements, to probe the often non-intuitive and highly nonlinear mechanics underlying morphogenesis, and to inform additional experiments.

Whereas the physical and computational models described thus far have provided significant advances in our understanding of the stresses and strains that generate tissue form, more recent work creating multi-scale models of morphogenesis have enabled the integration of additional levels of complexity by extending molecular- and protein-level signaling to tissue-level behaviors [70–74]. Ultimately, a complete picture of the physical mechanisms that drive morphogenesis requires models that capture the details of multicellular systems at multiple levels to understand the interactions and reciprocal feedback between genes, biochemistry, mechanics, and geometry which give rise to tissue architecture.

Remaining challenges

Even with all the advances made in the molecular, imaging, and computational tools described, additional progress is needed in a few key areas. Continued development of imaging modalities that enable rapid, non-destructive, high resolution scans with low phototoxicity, particularly *in vivo*, is essential for understanding morphogenetic cell and tissue movements. Efficient manipulation and visualization of large 4D datasets must improve as imaging modalities generate greater spatial and temporal resolution. With these larger data sets, there is a need for continued development and commercialization of custom image processing algorithms for automated alignment, segmentation, and cell-lineage tracking. In addition, efficient algorithms for surface fitting and rendering, as well as the generation of stable adaptively meshed FEM models are essential for these techniques to mature and gain widespread use. Lastly, challenges remain in defining appropriate constitutive descriptions for the tissues and integrating molecular models with the

mechanics. Understanding the multiscale interactions between the molecular signaling networks that regulate genetic programs and the microenvironmental cues will be key to elucidating the cellular behaviors and forces that drive morphogenesis.

Conclusions

Significant advances have been made in quantifying the kinematics and dynamics of morphogenesis within specific model systems. Newly developed imaging techniques permit the tracking of large populations of cells and yet also enable mapping cellular and tissue deformations with unprecedented temporal and spatial resolution. Genetic, molecular, and physical perturbations *in vivo* and in culture models, coupled with analytical and numerical analyses, continue to identify forces, molecular pathways, and developmental programs that are key to the (dys)regulation of tissue form. Continued development of experimental techniques and computational models that seek to integrate inter- and intracellular biological responses with cell and tissue mechanics will permit a more comprehensive understanding of the physical mechanisms of morphogenesis.

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Abbreviations

2D	two-dimensional
3D	three-dimensional
ECM	extracellular matrix
FEM	finite element method
GFP	green fluorescent protein
LeGO	lentiviral gene ontology
MSM	monolayer stress microscopy
OCT	optical coherence tomography
OPT	optical projection tomography
PIV	particle image velocimetry

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Highlights

- Physical forces and the mechanical environment generate tissue form
- A series of tools are used to quantify cellular motions, deformations, and forces
- Imaging and cell tracking advancements define the kinetics of morphogenesis
- Invertebrate and culture models elucidate cellular forces that drive morphogenesis
- Computational models describe and predict experimentally observed behaviors

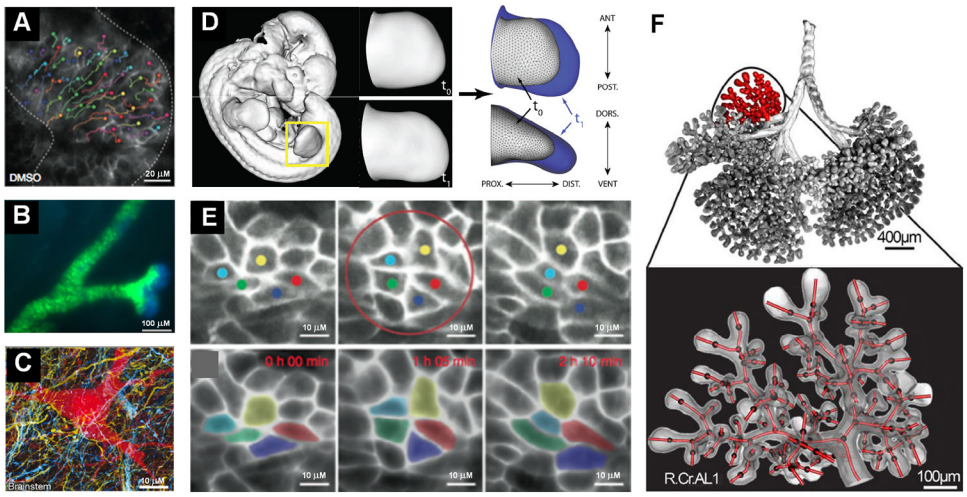

Figure 1.

Quantitative mapping of morphodynamics. Example strategies for labeling and tracking individual cells include using (A) membrane localized fluorophores [34], (B) chimeric embryos to track distinct cell populations [18], and (C) Cre/lox recombination techniques to track large numbers of individual cells [19]. Reconstruction of OPT imaging data produces (D) 3D surface renderings of morphology that can be used to compare initial limb bud morphologies (t_0) with those at later stages (t_1) [41]. Quantitative image processing reveals cellular rearrangements such as (E) rosette formation during kidney tube elongation [34] and (F) the branch morphology and lineage tree for ramified architectures such as the developing lung airways [42]. Images adapted with permission.

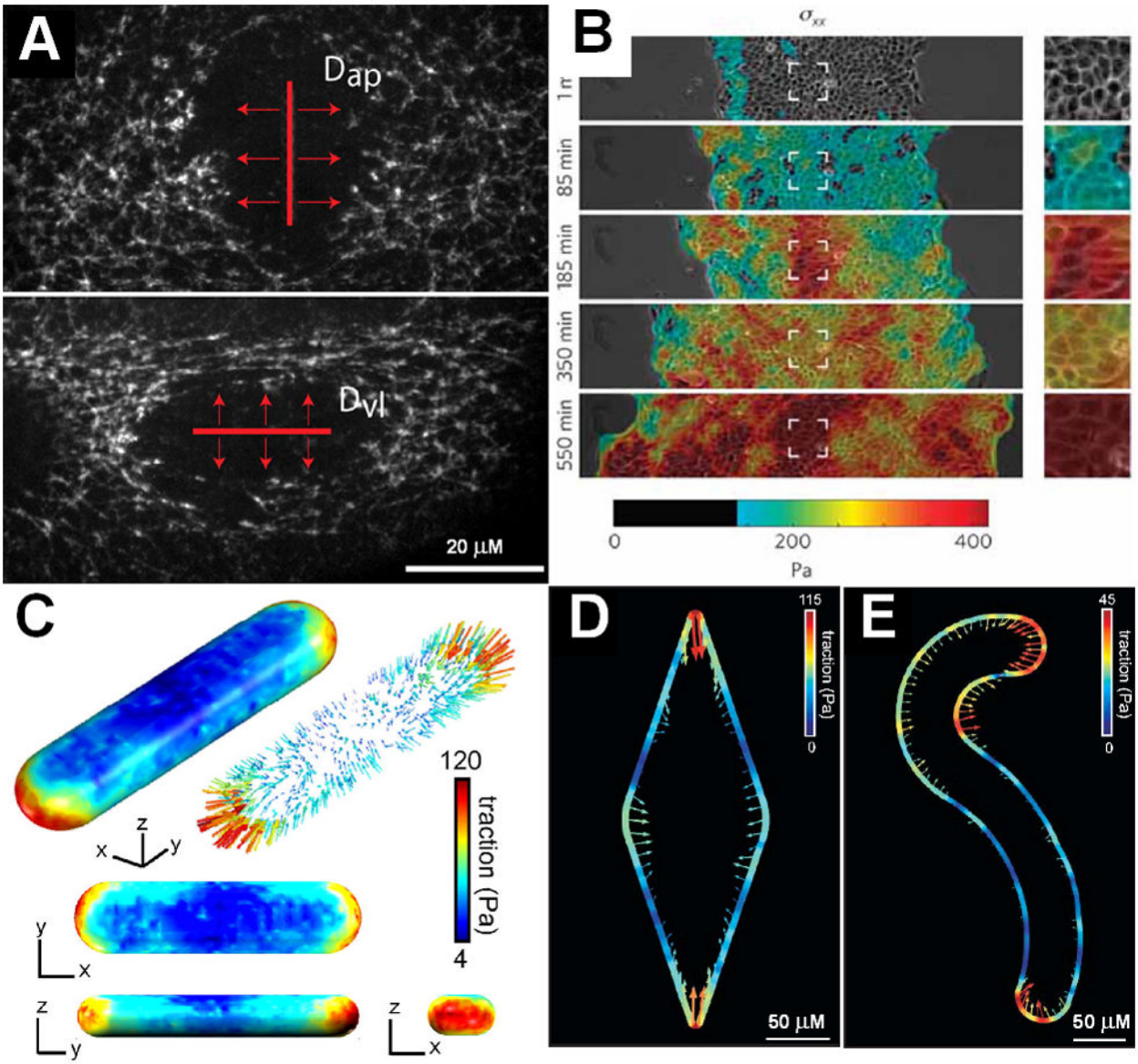

Figure 2.

Elucidating the forces that drive morphogenesis. (A) The overall wound geometry and the dynamics of the displacement of sub-cellular structures following laser ablation can be used to determine the nature and anisotropy of the stress field within a tissue, as shown with the displacement of GFP-tagged myosin networks in the ventral furrow of the *Drosophila* embryo [47]. Culture models are used to reduce the complexity of the microenvironment and provide more quantitative approaches to determine cellular and tissue-level forces. (B) Monolayer stress microscopy is used to define the stresses within a monolayer of cells collectively migrating on a polyacrylamide gel [55]. Similarly, micromolding techniques and 3D traction force microscopy can be combined to (C) calculate the interfacial traction forces of an epithelial tissue of (D, E) various geometries embedded in a collagen gel [60]. Images adapted with permission.

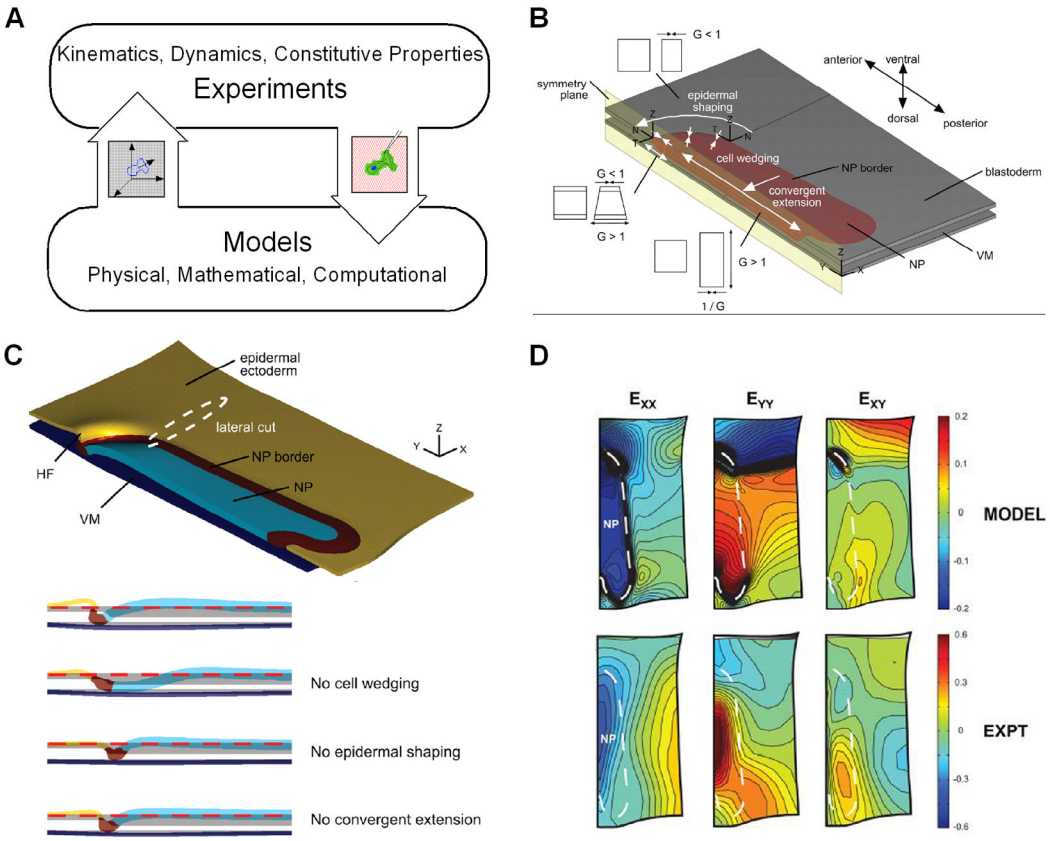

Figure 3.

(A) Physical, mathematical, and computational models work synergistically with experiments that measure cell and tissue mechanics to reveal physical mechanisms of morphogenesis and guide additional experiments. (B) Determining the mechanics of head-fold formation in the chicken embryo is one example of studies that integrate experimental data and computational models [16]. A finite element model was constructed with various cellular behaviors imposed on distinct tissue zones. (C) The imposed forces produce shape changes in the simulated tissue that mimic those seen in the embryo. (D) The computational model enables *in silico* hypothesis testing to determine the relative and combined roles for various cellular behaviors in the tissue deformations observed. Once a set of cellular behaviors that produce the overall tissue morphology are identified, the numerical results are further compared with experiments to verify that these physical mechanisms uniquely generate the observe tissue deformations. An example of one such verification is the comparison of the 2D Lagrangian transverse (E_{xx}) longitudinal (E_{yy}) and shear strain (E_{xy}) contours calculated from the head fold model and measured in the embryo. NP; neural plate. Images adapted with permission.