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Mapping morphogenesis and mechanics in embryo models

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Recent methodological advances in measurements of geometry and forces in the early embryo and its models are enabling a deeper understanding of the complex interplay of genetics, mechanics and geometry during development.

Over the last decade, in vitro stem cell models have provided a powerful approach to understanding the underlying principles of early mammalian development. This embryoid revolution was founded on two key insights: first, control over the initial geometry and cell number is crucial for self-organized patterning, and second, interaction between embryonic and extraembryonic tissues is required for morphogenesis of structures resembling the early embryo. Therefore, we find it useful to classify stem cell models according to their initial cell configuration, which then determines their complexity and the developmental processes they recapitulate.

The simplest systems involve only pluripotent stem cells (PSCs) that contribute to the embryo proper in vivo. Gastruloids are used to model gastrulation: the stage of embryonic development where the body plan is laid out and the three main lineages of the body — ectoderm, mesoderm and endoderm — are established. Gastruloids come in several variants displaying different degrees of patterning and morphogenesis. 2D micropatterned gastruloids start from a disc-shaped epithelium of PSCs on a substrate[1]. These initially form highly reproducible cell fate patterns with limited cell rearrangement but later undergo morphogenesis into a more complex multilayered structure[2]. In contrast, 3D gastruloids start from a spherical aggregate of PSCs that undergo spontaneous symmetry breaking accompanied by extensive cell rearrangement and subsequent axial elongation[3]. Other variants start with an epithelial cyst — that is, a hollow sphere that resembles the disc-shaped model after spontaneous[4] or explicit[5] symmetry breaking. Adding an initial differentiation step toward a particular lineage allows aspects of postgastrulation development to be recapitulated with similar approaches. For example, parts of ectodermal patterning and morphogenesis can be recapitulated starting from an ectodermal disc[6] and cyst[7], respectively.

More complex models combine embryonic and extraembryonic stem cell types. Blastoids combine small numbers of (extraembryonic) trophoblast stem cells (TSCs) and (embryonic) PSCs, which form a 3D spherical structure resembling the blastocyst[8]. Mouse ETS embryoids embed these cell types in extracellular matrix while ETX embryoids include extraembryonic endoderm; in both cases a cylindrical epithelial structure resembling the mouse embryo at the onset of gastrulation arises[9]. ETiX embryos include extraembryonic endoderm-like cells induced by transcription factor overexpression and can develop much further, completing gastrulation and continuing into organogenesis[9]. A similar strategy was recently used to model tissue interactions in the peri-implantation human embryo[10].

In our opinion, each stem cell model should be viewed as simplifying development in specific ways that enable answering questions that are difficult to address in the embryo. Therefore, differences from in vivo development can be useful features rather than shortcomings. By comparing different models and leveraging their individual technical advantages, developmental mechanisms can be revealed that are obscured by the complexity of the full embryo. For example, embryo models have demonstrated how patterning of embryonic tissue occurs in the absence of morphogenesis or extraembryonic tissues and have provided insight into patterning mechanisms by varying tissue size and shape. We believe the next challenge lies in understanding how patterning, mechanics and morphogenesis are coupled; certainly, embryo models will play a key role in this by enabling comparison of different tissue configurations while providing unique experimental advantages. Here we describe recent advances in methods for understanding the disparate spatial organizations of embryo models at different scales, with an emphasis on how tissue flow and geometry can be used to infer tissue mechanics.

Relating flow and forces during embryogenesis

Morphogenesis can be described very generally by tissue flow, that is, the local rate of displacement. Tissue flow can be obtained from imaging data directly at the pixel level using particle image velocimetry or optical flow[11]. In a first application of such techniques to embryo models, optical flow was used to demonstrate that the endodermal cap forms by collective cell migration in 3D gastruloids[12] (Fig. 1a,b). Alternatively, tissue flow can be obtained by cell tracking, which provides more information about the cellular-level movements that underlie the overall tissue shape change. Recent improvements to cell tracking include machine learning approaches that improve segmentation and efficiently handle very large datasets by automatically discriminating spatiotemporal features of cell lineages and cell division events[13,14]. These methods were shown to be very effective at analyzing mouse gastrulation and can be anticipated to soon find application in studying embryo models.

Tissue flow is generated by mechanical forces, with the relationship between flow and forces depending on the mechanical properties of the tissue. The tissue can generally be considered as an active viscoelastic material[15], meaning that it has both fluid- and solid-like properties and is therefore characterized by both viscosity and elasticity. Given a model of tissue mechanics, one can infer forces from the measured tissue flow in the same way that Hooke's law allows one to relate the deformation of a spring to the applied force given the spring constant. In a beautiful application of this, tissue flow in chick gastrulation was discovered to behave as viscous flow driven by a ring of tension on the embryo margin that is not simply downstream of embryonic patterning

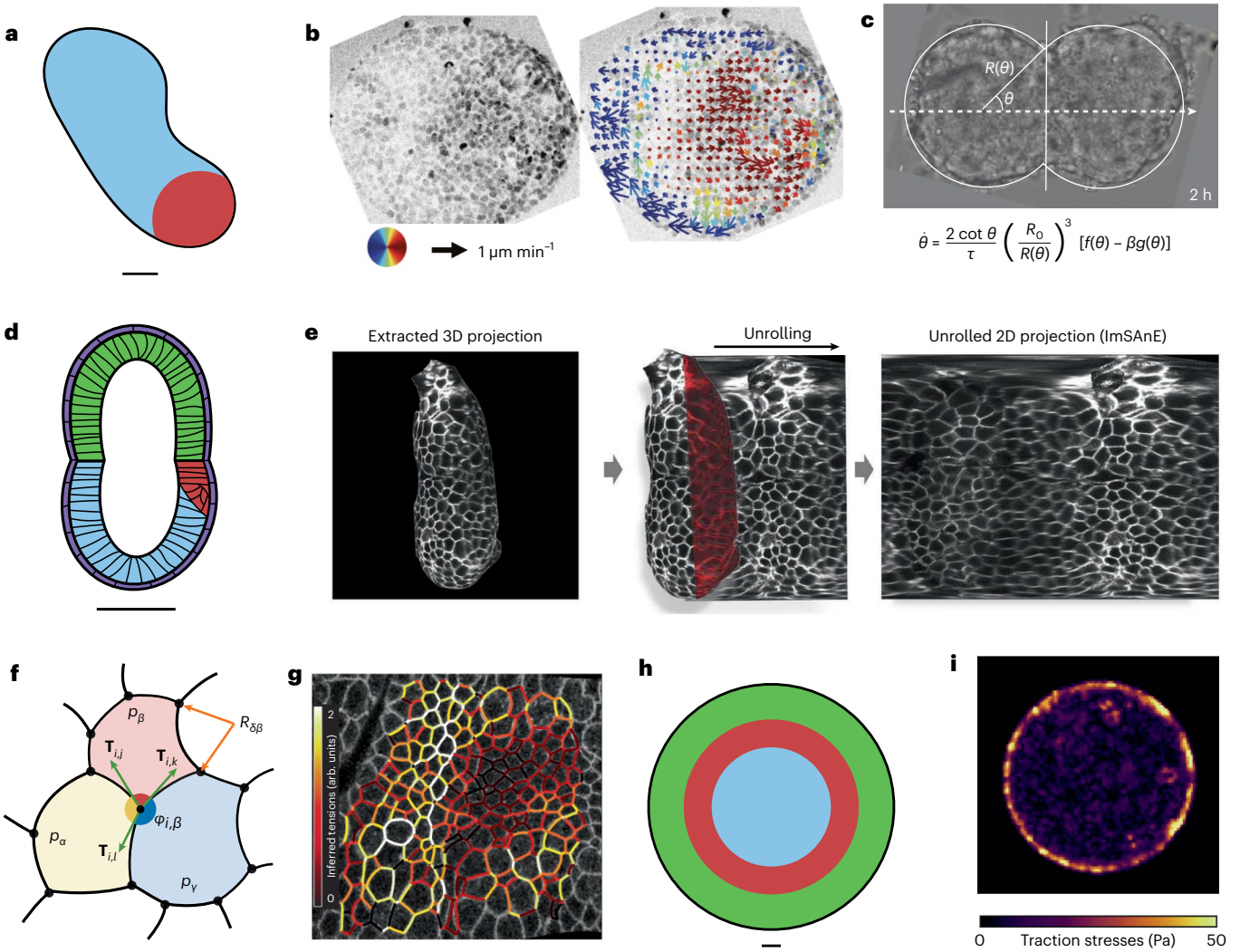

Fig. 1 | Methods to study morphogenesis and mechanics in embryo models. **a**, Schematic of 3D gastruloid[38]. In all schematics, red indicates brachyury-positive primitive streak-like cells, blue pluripotent cells, green extraembryonic cells. Scale bars in **a**,**d**,**h**, 50 μm. **b**, Tissue flow in 3D gastruloid expressing brachyury reporter (dark)[12] **c**, Fusion of mouse embryonic stem cell aggregates; equation describes expected time evolution and can be fitted to obtain the τ and β parameters, which are proportional to the ratios of shear viscosity and shear modulus, respectively, to surface tension[22]. **d**, Schematic of ETX embryoid. **e**, Tissue cartography using ImSAnE (Image Surface Analysis Environment)[24] for the inner epithelium, consisting of epiblast and extraembryonic ectoderm[25]. **f**, In mechanical equilibrium, interfacial tensions **T** and cellular pressures p in an epithelium can be inferred from vertex angles φ and membrane curvature radius R. **g**, Inferred membrane tensions from cell geometry in *Drosophila* lateral ectoderm[29]. **h**, Schematic of 2D gastruloid. **i**, Map of traction stresses in 2D gastruloid[32].

but is part of a coupled mechanochemical patterning system[16]. Similar applications can be expected for embryoids in the future.

Force inference from cell and tissue geometry is powerful because it is global and high throughput, but it rests on assumptions about the mechanical properties of the tissue and only provides relative forces. When based on tissue flow, it also lacks cellular resolution. Inferred forces must therefore be verified with other methods. Deformation in response to laser cutting can confirm relative tensions but is destructive, instantaneous and low throughput. Genetically encoded fluorescent force sensors exist but may be difficult to relate to specific mechanical forces on the cell[17]. To address this, methods were developed in the last decade to obtain absolute mechanical stresses over time from deformation of injected liquid droplets or gel beads with known mechanical properties[18,19]. These methods will be useful in, for example, determining how the mechanical properties of 3D gastruloids change as they differentiate.

Coarse-grained approaches for large aggregates

An advantage of embryo models is throughput: the ability to produce thousands of structures per experiment. However, general

methods to measure tissue flow and forces require high spatial- and temporal-resolution imaging. For high throughput screening of large structures such as 3D gastruloids, this is not feasible, and a coarser description of shape is appropriate. For this purpose, methods were recently developed to capture large-scale features of the geometry such as the volume, area, eccentricity or number of lobes from low-resolution bright-field images[20,21]. Taking this one step further, a technique was developed to obtain mechanical properties by measuring the contact angle during the fusion of homogeneous aggregates over time. This was applied to mouse embryonic stem cell aggregates that represent the initial state of 3D gastruloids[22] (Fig. 1c).

Tissue layers and epithelial morphogenesis

The downside of high-throughput methods is that outlines of structures in bright-field images contain limited information. High-resolution measurements of tissue flow in three dimensions contain much information but may be hard to interpret. Analysis can be simplified by methods adapted to the organization of the embryo. The early embryo and many of its stem cell models are largely organized into collections of two-dimensional, mostly epithelial, structures. For example, ETS embryoids consist of three epithelia: extraembryonic ectoderm, epiblast and extraembryonic endoderm. Much insight can therefore be gained by decomposing the data along these layers and determining which cellular processes drive their morphogenesis. This poses a challenge, especially for curved time-dependent tissue surfaces. To address this, tissue cartography methods were developed that detect these surfaces and produce two-dimensional projections analogous to maps of earth[23,24]. These maps simplify visualization and enable efficient 2D analysis methods for segmentation and tracking; this was recently applied to analyze cell contact angles in ETX embryos[25] (Fig. 1d,e). A major step forward in tissue cartography was the development of TubULAR, a method to globally analyze deformation of complex dynamic tissue geometries[26]. TubULAR establishes a material frame of reference in which cell movement is minimized and decomposes tissue flow into different in-plane and out-of-plane components, greatly simplifying dissection of epithelial morphogenesis into its constituent cell behaviors such as rearrangement, shape change and proliferation.

Tissue cartography enables application of powerful 2D methods to analyze epithelial morphogenesis. On the basis of membrane markers, cell junctions can be segmented with relative ease to analyze cell shape and neighbor exchange. At least near the apical surface, epithelial mechanics is often dominated by junctional tensions and pressure. Moreover, morphogenesis typically occurs on time scales much longer than the viscoelastic relaxation of the tissue, implying that epithelial morphogenesis can be treated as a slowly changing mechanical equilibrium. Together this means junctional tension and cell pressures can be inferred from cell shape with simple force balance equations. The first force inference methods approximated cell interfaces as straight, reducing the amount of information in the shape and making the inference sensitive to noise[27,28]. Recent improvements address this noise sensitivity by further constraining forces by measured membrane curvature and directly fitting cell geometries that are consistent with the assumption of mechanical equilibrium[29,30] (Fig. 1f,g). An exciting frontier is to combine such approaches with optogenetic force manipulation to understand morphogenesis in embryo models[31].

For 2D embryo models that are attached to a substrate, such as micropatterned gastruloids, in addition to inference of intercellular apical forces, traction forces on the substrate can be measured with traction force microscopy. Moreover, substrate stiffness can be modulated. This was used to show that increased contractility on gastruloid boundaries[32] and substrate stiffness may modulate cell signaling activity and downstream cell fate patterning[33] (Fig. 1h,i). It provides a future opportunity to relate basal traction forces to apical intercellular forces during pattern formation and subsequent morphogenesis.

Small aggregates and early embryoids

Before forming large epithelial structures, embryo models typically combine small numbers of embryonic and extraembryonic cells in suspension. The initial dynamics of these cells as they rearrange into organized structures is of great interest. Understanding this benefits from analysis of individual cell shapes and interfaces in 3D with high accuracy and single-cell measurements of mechanical properties.

Recent advances were made for subcellular segmentation of small embryos, including early mouse and ascidian embryos, with the goal of more accurate inference of mechanics. Delaunay-watershed was designed to avoid introducing biases (for example, on angles) that affect force inference[34]. A different approach generalizes the 2D approach for enforcing consistency of the segmentation with mechanical equilibrium to 3D[35]. Both methods then use this to improve previous 3D force inference[36]. To verify predictions, tension of – and adhesion between – individual cells can be measured directly with micropipette aspiration. This was previously done for the mouse blastocyst and recently applied to measure the differential adhesion between cell types in ETX embryoids[25,37].

Outlook: interplay of patterning and morphogenesis

Traditionally, tissue patterning and morphogenesis have largely been studied in separation. However, it is now clear that these processes are intricately linked through an interplay between mechanics, cell signaling and gene expression within the dynamically changing geometry of the embryo. Recent methodological advances have substantially improved our ability to relate mechanics to tissue geometry in embryo models. One limitation of current methods is their inability to address interactions between tissue layers, and we view this as an important future direction. For example, epithelial force inference methods have focused on apical force balance, and it will be important to determine how these are coupled to basal forces on the underlying extracellular matrix and other tissues – for example, in the case of the epiblast and hypoblast. Furthermore, limited attention has been paid to the mechanical interaction between the mesenchymal cell populations arising in gastrulation and the surrounding epithelia. As our toolkit expands, we believe that comparison between real embryos and stem cell models that decouple morphogenesis and patterning to varying degrees will enable confrontation of the complexity of this interplay in vitro and in vivo.

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Competing interests

The authors declare no competing interests.