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Mechanics as a means of information propagation in development

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Abstract

New research demonstrates that mechanics can serve as a means of information propagation in developing embryos. Historically, the study of embryonic development has had a dichotomy between morphogens and pattern formation on the one hand and morphogenesis and mechanics on the other. Secreted signals are the preeminent means of information propagation between cells and used to control cell fate, while physical forces act downstream or in parallel to shape tissue morphogenesis. However, recent work has blurred this division of function by demonstrating that mechanics can serve as a means of information propagation. Adhesive or repulsive interactions can propagate through a tissue as a wave. These waves are rapid and directional and can be used to control the flux of cells through a developmental trajectory. Here, we review two examples in which mechanics both guides and mediates morphogenesis and two examples in which mechanics intertwines with morphogens to regulate cell fate.

Keywords

Drosophila; zebrafish; chick; intestinal stem cells; tailbud; cell migration; gastrulation; myosin; cadherin; integrin; nodal; bmp; sonic hedgehog; eve1

1. Introduction

Embryonic pattern formation is the process by which different cell types and cell behaviors are appropriately positioned in space and time in the developing embryo. In response to signals, cells adopt specific behaviors such as to proliferate, change shape, or migrate, so as to generate the correct proportion and localization of cell types to generate a viable organism. The discovery of embryonic organizers and subsequent identification of morphogens are the seminal breakthroughs in developmental biology. Morphogens are typically secreted molecules that diffuse from an organizer or source to form a gradient. They specify at least two cell fates above a ground state in a concentration-dependent manner.^[1] The “French Flag” model illustrates how the morphogen concentration creates positional information by specifying the blue, white and red stripes of the flag as a function of distance from the source of the morphogen. There are now numerous in vivo examples of

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morphogens and the mechanisms that establish and interpret these signaling gradients to specify embryonic pattern.^[2]

Morphogenesis is the change in form of the embryo in response to pattern formation. This process largely relies upon the regulated activity of the cytoskeleton and its associated motor proteins to generate force, as well as the processes of cell-cell adhesion and cell adhesion to the extracellular matrix to mechanically couple neighboring cells.^[3] The advent of experimental embryology in the 19th century ushered in the modern era of developmental biology and the study of morphogenesis while technological innovations in genetics and microscopy have revolutionized the field in recent decades.

Heuristically, pattern formation and morphogenesis can be considered separately for the sake of simplification. However, it is clear that pattern formation may proceed morphogenesis and that another round of pattern formation may occur after a morphogenic event as cells are brought into proximity to new neighboring cells. Thus, the two processes are clearly inter-dependent. Pattern formation and morphogenesis may also occur concurrently, and in some cases, the conceptual boundary between the two processes is no longer clear. Here, we review four such cases in which mechanical processes, normally associated with morphogenesis, are intertwined with pattern formation. These developmental processes include invagination of the *Drosophila* endoderm, zebrafish body elongation, chick gut development and cell fate specification in the zebrafish embryonic organizer.

2. Examples of purely mechanical information propagation

2.1. Synchronized pushing and pulling during *Drosophila* endoderm invagination

The gastrulating *Drosophila* embryo is a highly coupled mechanical system.^[4] It starts as a monolayer epithelium encapsulating an oblong yolk (Figure 1A). Endoderm invagination at the posterior tip of the embryo pulls on the germ band inducing a tissue scale (over 100 microns) anterior-posterior tension gradient.^[5,6] This tension promotes germband elongation by orienting junctional remodeling in convergent extension. However, according to Newton's Third Law, the germband and the more anterior cells must also exert tension on the endoderm. Thus, the question is how does the endoderm accomplish directional invagination?

Recent work from Bailles et al., has revealed that the invaginating domain can be divided into two distinct regions, the endoderm primordium to the posterior and the propagation region immediately anterior, which use different mechanisms for invagination.^[7] The primordium invaginates first in response to a secreted signal, *folded gastrulation* (*fog*), which induces myosin recruitment, apical constriction, and simultaneous invagination of the entire region. In contrast, the propagation region subsequently invaginates one row of cells at a time through an advancing furrow. Myosin recruitment occurs in a propagating wavefront immediately ahead of the furrow. The velocity of this wavefront is 2.2 microns per minute or about one cell length every 3 minutes. ^[7] Importantly, this recruitment is not controlled by *fog* as changing the dosage of *fog* has no effect on wavefront propagation (provided the initial invagination of the primordium remains intact). While in principle a gradient of some

other molecule could be used to control the timing of myosin recruitment, the known mechanical sensitivity of myosin makes a purely mechanical model plausible.^[8,9]

Bailles et al., proposed a mechanical relay in which the morphological changes of invagination exert forces on the cells anterior to the furrow leading to myosin recruitment, apical constriction, and furrow progression (Figure 1A). To elaborate further, the cytoplasm is incompressible, so when a cell contracts apically it must expand basally. Thus, the forces exerted on neighboring cells at the lip of the invagination, i.e. pulling in one plane and pushing in another, induce these cells to undergo apical expansion and basal compression. Additionally, the yolk is incompressible so the inward displacement of the invaginated cells produces hydrodynamic forces that elevate cells outside the invagination.^[10] These morphological features are indeed observed in the cells immediately anterior to the furrow, prior to myosin activation. The elevation of the cells ahead of the furrow brings them into contact with the vitelline membrane that surrounds the embryo. Importantly, propagation region cells express an apically localized integrin that adheres to the vitelline membrane. Activation of the integrin in turn recruits myosin, triggering apical constriction and thus completing the mechanical cycle.

The point of contact with the vitelline membrane provides an anchor to enable force propagation during endoderm invagination and germband extension.^[7,11] The requirement for the anchor point is demonstrated by analyzing embryos lacking the integrin. In wild-type embryos, cells in the propagation zone ahead of the furrow move anteriorly with a constant slow velocity^[7] indicating that these cells are experiencing a small net force directed away from the furrow. However, integrin knockdown embryos have an altered pattern of cell motion. Before the onset of invagination cell velocities are akin to those of wild-type embryos. Once the primordium starts to invaginate, cell velocity in the propagation zone rises rapidly to approximately 8 microns per minute. This is three to four times the speed of furrow propagation. Consequently, these embryos have a shallow initial invagination without a steep furrow. These results indicate that adhesion can promote invagination by resisting opposing forces and providing an anchor for myosin to pull against. Moreover, integrin mutants have improper twisting of the germ band during its extension indicating that formation of the anchor is also important for other tissues.^[11,12]

Interestingly, endoderm invagination does recover in integrin knockdown embryos.^[7,12] After a delay, the invagination deepens via an unknown mechanism. At this point, cells in the propagation zone reverse direction and move at high velocity to the posterior and into the invagination. How the reversal in the net direction of force is achieved is unknown, but the flow of cells into the furrow is presumably aided by a lack of friction in integrin knockdowns that allows the cells to slide freely against the vitelline membrane. Consistent with this hypothesis, integrin mutants have minimal myosin activation in the propagation region.^[7] These observations suggest there are at least two practical routes to achieve endoderm invagination in *Drosophila*. The first route is the wild-type mechanism in which the cells are transiently anchored and the furrow advances, while the second route is used in the integrin knockdown in which the furrow is largely fixed and the cells move. It will be of interest to use enhanced modeling and analysis of various genetically altered *Drosophila*

embryos subject to different force loadings to explore the strengths and weaknesses of these mechanisms.

2.2. The wisdom of cell crowding during early spinal column elongation

Another example of a mechanical relay is in the zebrafish tailbud. The tailbud is the posterior leading edge of the elongating vertebrate embryo and contains progenitors of skeletal muscle as well as the nerve, bone and cartilage of the spinal column. As the embryo elongates posteriorly, the tailbud exhibits a constant flux of migrating cells (Figure 1B). The dorsal posterior tailbud contains a pool of neuromesodermal progenitor cells (NMPs) that support body elongation by contributing cells to both the somites and the spinal cord. As the posterior neural tube extends, cells flow into the dorsal posterior tailbud from the neural tube in a progressive, orderly fashion. Cells in the very posterior tip of the embryo that are fated to become mesoderm undergo an epithelial-mesenchymal transition (EMT) and migrate ventrally and begin to express mesodermal genes such as *tbx16* and *mesogenin*. Cells engage in chaotic, disorderly motions in this region before joining the left and right presomitic mesoderm. An efficient posterior flow of cells through the dorsal tailbud is necessary to achieve full body elongation, and the disordered flow in the ventral posterior tailbud ensures bilaterally symmetric elongation.^[13] However, this rapid flow limits the amount of time cells can experience the signals that maintain tailbud elongation. A recent paper from Das, Julich, Schwendinger-Schreck et al., focusing on BMP signaling explored this dichotomy.

The posterior tip of the tailbud contains the tail organizer that expresses *bmp 2/4* and the transcription factor *even skipped (eve1)* and has tail inducing activities.^[14–16] A positive feedback loop exists between Bmps and *eve1* where Bmp signaling promotes *eve1* expression and *eve1* promotes transcription of *bmp2* and *bmp4*.^[17] Importantly, this is a short-range feedback loop. Both transcription of *bmp4* and *eve1*, as well as phosphorylated-SMAD, indicative of Bmp signaling, are all tightly localized to the very posterior tailbud. However, breaking this feedback by applying a pharmacological inhibitor of BMP signaling, overexpressing *eve1* specifically in the tailbud or both, has short- and long-range effects on cell motion. Locally, there is decreased mean cell velocity and cell motion is more disordered. In the posterior neural tube, among cells that were yet to be exposed to BMP or express *eve1*, these perturbations have no effect on mean cell velocity but did decrease ordered cell motion. Similar to Bmp signaling, there are high levels of both Wnt and Fgf signaling in the posterior tailbud, and these pathways are required for normal body elongation. Expression of Wnt and Fgf target genes is altered by blocking Bmp signaling, but inhibition of either Wnt or Fgf signaling does not reproduce the long-range effects of blocking Bmp signaling. Thus, it is unlikely that indirect alteration of Wnt or Fgf signaling causes the long-range modulation in cell movement in the posterior neural tube.

Das, Julich, Schwendinger-Schreck et al., explored the possibility that a mechanical relay mediates this long-range modulation in the tailbud. They reasoned that the increased disorder in the posterior tailbud could create a traffic jam that, like a pressure wave, would propagate anteriorly approximately 10 cell diameters away into the posterior neural tube (Figure 1B). Similar waves have recently been observed in high density Dictyostelium

cultures.^[18] Das, Julich, Schwendinger-Schreck et al., explored this possibility using a 3D computational model of the tailbud. This model included adhesive and repulsive cell interactions and a tendency for cells to align their velocity with those of their neighbors. The experimental perturbations of BMP and *eve1* activity were modeled as an increase in cell repulsion in a fraction of cells in the tail organizer. This *in silico* perturbation recapitulated the reduction in ordered cell motion seen in the *in vivo* perturbations via BMP inhibition and *eve1* overexpression. The computer simulation also made new predictions. While cells in the posterior neural tube move in an anterior to posterior direction in wild-type embryos, after the *in silico* perturbation some cells transiently reverse direction and move towards the anterior. This is similar to the propagation of a pressure wave through sand. This prediction was confirmed *in vivo*.

A second model prediction was that upon the onset of an acute perturbation the disturbance in cell velocity would propagate anteriorly through the neural tube as a wavefront. The wavefront gradually dissipates due to the countervailing anterior to posterior flow of cells. The second model prediction was more challenging to test because it required a spatially and temporally controlled perturbation *in vivo*. Das, Julich, Schwendinger-Schreck et al., generated such a perturbation by first mounting embryos, which mis-express *eve1* in the tailbud, on a microscope slide and then adding a pharmacological Bmp inhibitor at the beginning of the timelapse. By capturing the onset of Bmp inhibition during the timelapse, a propagating disturbance in the cell velocity field was detected. The estimated speed of the wave was 1 micron per minute at 18°C, similar to average cell speed in the posterior neural tube. A further test was to use a more mechanical perturbation by overexpressing a myosin activator in the posterior tailbud to increase cytoskeletal contractility and perturb cell migration within the tail organizer. This perturbation again created a long-range perturbation in cell migration in the posterior neural tube far from the cells expressing the transgene.

The tailbud mechanical relay was proposed to match the flow of cells into the tail organizer to the flow of cells out of the organizer. This efficient movement of cells through the tailbud is required for full body elongation. A major outstanding question is the nature of the relay. The modeling is consistent with contact inhibition of locomotion emerging either passively by volume exclusion or via rapid cell signaling (i.e. independent of transcription and translation). For example, a recent study of confluent 2D cell culture identified a role for extracellular signal-regulated kinase (ERK) signaling in a mechanical cell to cell relay that directs collective cell migration.^[19] The mechanical relay may also serve as a homeostatic mechanism to ensure maintenance of the NMP pool. There is crosstalk between BMP and Wnt and FGF, and the latter two signals are known to regulate the choice between neuronal and mesodermal fate and the initiation of EMT in the tailbud. For these decisions to be made correctly cells must accumulate adequate levels of signaling while transiting the tailbud. In the presence of a robust BMP-*eve1* positive feedback loop, cells are able to move efficiently. If the feedback loop is weakened cell velocity drops, increasing residence time in the niche. This allows the cells time to accumulate adequate signaling for themselves and to secrete sufficient BMP to kickstart the feedback loop in their successor cells. More quantitative measurements of the dynamics of cell signaling and transcription factor expression will help flesh out this model.

3. Mechanics as a modulator of morphogen signaling

3.1. Intestine development: from tissue folds to signaling gradients

While mechanics can be used to convey information directly, it can also exhibit crosstalk with morphogen signaling. One example is how the concentration gradient of Sonic Hedgehog is formed during chick intestine morphogenesis (Figure 2A). The adult intestine has a distinct structure of crypts and villi with stem cells residing deep in the crypts while differentiated cells populate the villi.^[20] Differentiation of epithelial villi cells is promoted by a signaling loop between the epithelium and a small pool of mesenchymal cells underlying the tip of the villi known as the “villus cluster”. The villus epithelium secretes Sonic Hedgehog, which induces BMP expression in the villus cluster. In turn, BMP signaling in the villus confines Wnt signaling to the crypt where it is required for stem cell maintenance.^[21]

Establishment of the adult morphology and distribution of cell types along the crypt-villus axis is entwined. Early in development, the intestine is a smooth epithelial tube comprising a homogenous pool of stem-like cells overlying a mesenchyme. The epithelium expresses Sonic Hedgehog, but the concentration is insufficient to robustly activate villus cluster genes.^[22] As the intestine develops, continued epithelial proliferation in the context of less proliferative layers of smooth muscle constrains the epithelium and causes it to buckle, first into longitudinal ridges then into a zigzag pattern.^[23] It is at this point that stem cells become restricted to the base of the ridges. The narrow grooves at the apex of the ridges create a pocket in which the underlying mesenchyme is exposed to a steep gradient of sonic hedgehog (Figure 2A). At the very tip of the ridge, the concentration is high enough to robustly activate genes expressed in the villus cluster. Signaling then adopts the feedback between Bmp and Wnt signaling that maintains intestinal homeostasis in the adult. The final remodeling of the zigzag ridges into crypts and villi requires a cessation of cell division specifically at the tips of the ridges.^[23] BMP signaling from the villus cluster suppresses cell division at the tips. Thus, in the intestine, the mechanics shapes the morphogen gradients and the morphogens shape the mechanics.

3.2. Mechanics and cell fate specification in the zebrafish embryonic organizer

The prechordal plate is a compact mass of mesodermal cells derived from the embryonic shield (the zebrafish equivalent to Spemann’s organizer). As the mesoderm and endoderm are induced prior to gastrulation, cells within the organizer that maintain a high level of Nodal signaling will express *gooseoid* and differentiate as mesoderm while a smaller number of cells with lower levels of Nodal signaling express *sox17* and become endoderm.^[24,25] Recent work has revealed that this cell fate decision is shaped by a kinetic proofreading circuit involving both Nodal signaling and cell-cell adhesion (Figure 2B).^[24] To elaborate, there is a positive feedback loop between Nodal signaling and cell adhesion. Nodal signaling increases the area and duration of cell-cell contacts by increasing expression of E-Cadherin. In turn, cell-cell adhesions are sites of enhanced Nodal signaling as indicated by increased accumulation of fluorescently labeled Nodal at cell-cell contacts. However, *in vivo* the level of *gooseoid* expression does not correlate with the number of cell contacts but rather with the duration of contacts. A dependence on duration rather than concentration

is characteristic of kinetic proofreading. To explore this idea further, Barone et al., constructed a detailed stochastic model of the system using mostly experimentally determined rate constants. Kinetic proofreading was encoded in the model as a delay between cell-cell junction formation and the potentiation of Nodal signaling. Models with the proofreading exhibited bistability while those without only had the high Nodal steady state. Thus, based on these *in silico* models, mechanical proofreading helps to stabilize the choice between mesoderm and endoderm cell fate.

4. Conclusions and outlook

Mechanics is not just part of the physical process of shaping tissues but also a unique means of cell communication. Morphogen signals are controlled by the dynamics of diffusion. They generally spread isotropically and can be detected by any cell in the vicinity of a source expressing the morphogen receptor. Mechanical information propagates through a soft matter, so the cells likely need to be sufficiently packed or connected indirectly by an extracellular matrix to relay the mechanical information. Because the embryo is overdamped, i.e. very soft, propagation of mechanical information likely requires an active relay from cell to cell involving the cytoskeleton and hydrolysis of ATP. Discontinuities in the mechanical properties of the tissue could influence which cells receive the mechanical information and produce anisotropic propagation. The speed and distance of the wavefronts in the *Drosophila* embryo and the zebrafish tailbud are akin to what can be achieved with a morphogen. Interestingly, in both contexts the velocity of the wavefront is approximately the same as the cell movement velocity. More work is needed to determine whether there is a fundamental link between the two processes. For example, in the zebrafish tailbud the mechanical signal manifests as a wave of altered cell migration behaviors and propagation may be different in tissues with lower cellular motility. Further research will likely uncover more examples of mechanics acting alone or in concert with morphogens to convey information and orchestrate animal development.

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Figure 1. Examples of biological mechanical relays.

(**A**) Diagram of a *Drosophila* embryo during gastrulation. The endoderm invaginates through a propagating furrow. Apical contraction and basal expansion of invaginated cells pulls and pushes, respectively, on cells at the lip of the furrow (grey) inducing apical expansion, basal compression and myosin recruitment. This pushing and pulling relay drives directional propagation of the furrow. (**B**) Diagram of zebrafish tailbud. Perturbations of the flow of cells in the BMP positive region ripple anteriorly into the posterior neural tube.

Figure 2. Examples of mechanics regulating morphogens.

(**A**) Diagram of developing chick intestine. Bending of the tissue creates a pocket with the high concentration of sonic hedgehog required to activate villus cluster genes and confine stem cells to the base of villus. (**B**) Schematic of kinetic proofreading in the zebrafish prechordal plate. Prolonged cell-cell contact promotes nodal signaling and nodal increases expression of E-cadherin, but the system resets to baseline if cell-cell contact is lost.