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Emergent morphogenesis: elastic mechanics of a self-deforming tissue

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Abstract

Multicellular organisms are generated by coordinated cell movements during morphogenesis. Convergent extension is a key tissue movement that organizes mesoderm, ectoderm, and endoderm in vertebrate embryos. The goals of researchers studying convergent extension, and morphogenesis in general, include understanding the molecular pathways that control cell identity, establish fields of cell types, and regulate cell behaviors. Cell identity, the size and boundaries of tissues, and the behaviors exhibited by those cells shape the developing embryo; however, there is a fundamental gap between understanding the molecular pathways that control processes within single cells and understanding how cells work together to assemble multi-cellular structures. Theoretical and experimental biomechanics of embryonic tissues are increasingly being used to bridge that gap. The efforts to map molecular pathways and the mechanical processes underlying morphogenesis are crucial to understanding: 1) the source of birth defects, 2) the formation of tumors and progression of cancer, and 3) basic principles of tissue engineering. In this paper, we first review the process of tissue convergent-extension of the vertebrate axis and then review models used to study the self-organizing movements from a mechanical perspective. We conclude by presenting a relatively simple "wedge-model" that exhibits key emergent properties of convergent extension such as the coupling between tissue stiffness, cell intercalation forces, and tissue elongation forces.

Keywords

cell shape and tissue mechanics; modeling; in silico; computer simulation; gastrulation; frog; convergence and extension; convergent extension

Introduction

Cell- and tissue-scale mechanics are thought to play a major role in shaping biological forms such as the body plan of animals as well as internal structures such as bones and organs (Cowin, 2000; Wozniak and Chen, 2009). Mechanics can play three distinct roles in developing multicellular structures: 1) *Multicellular-Integration* -mechanical integration at the tissue level coordinates force production and viscoelastic material properties of tissues to dictate the direction and speed of tissue movements as structures are sculpted (Beyer and Meyer-Hermann, 2009; Ghysels, Samaey et al., 2009; Kumar and Weaver, 2009; Davidson, Von Dassow et al.,

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in press), 2) *Intracellular-Cell Integration* - mechanical integration of intracellular force generation with the local micro-mechanical environment to direct intracellular molecular-mechanical processes that manifest as a cell behavior (Lecuit, 2008; Xia, Thodeti et al., 2008; Pouille, Ahmadi et al., 2009; Vogel and Sheetz, 2009), and, 3) *Intracellular-Gene Integration* -mechanical integration of the cell, the micro-mechanical environment, and gene regulatory networks to direct cell differentiation (Chen, Mrksich et al., 1997; Engler, Sen et al., 2006; Engler, Sweeney et al., 2007; Lopez, Mouw et al., 2008). The last two roles of mechanics, integrating aspects of intracellular force generation with local topographic and signaling cues, are typically grouped within the term "mechanotransduction" but it is useful to separate processes involved in mechanical "feedback" from those mediating mechanical "positional information".

Historically, the goals of developmental biology include understanding the molecular genetic as well as the mechanical principles of embryonic morphogenesis. Research on invertebrate model organisms such as *Caenorhabditis elegans* (roundworm) and *Drosophila melanogaster* (fly) with their rapid development and tractable genomic organization have led the way toward elucidating the molecular pathways that regulate development. These model organisms have also been indispensable in connecting molecular pathways to specific cell behaviors, for instance, revealing the cell biology that underlies coordinated movements of epithelial cells during large-scale morphogenetic movements that build grooves, elongate tissues, and enclose the embryo (Hardin and Walston, 2004; Lecuit and Lenne, 2007; Quintin, Gally et al., 2008). Vertebrate model organisms ranging from zebrafish, frog, chicken, and mouse complement invertebrate studies and extend them to anamniotes, amniotes, and mammals. Furthermore, molecular analysis of cell behaviors during vertebrate development can draw on research carried out with cultured cell lines derived from tumors and primary adult tissues.

We focus this review on convergent extension, a single example of morphogenetic tissue movement, because it is one of the earliest and largest movements during vertebrate morphogenesis (Keller, 2002). All vertebrate embryos that have been studied in any detail exhibit this movement. Convergent extension can occur within epithelial or mesenchymal cell types is one of the best characterized morphogenetic movements on both the cellular and molecular level. Thus convergent extension provides a useful example for engineers to consider as they seek to control cell behaviors and shape novel tissues. Theoretical models of morphogenesis strive to understand how molecular pathways control cellular mechanics (the featured topic in this issue). For many years, discussions on the mechanics of morphogenesis were purely theoretical; qualitative or "word models" prevailed to explain many phenomena. However, as the interconnected molecular pathways operating during morphogenesis have been mapped, and high-powered computing devices have become more accessible, discussions turned to more quantitative models. Theoretical models, computer simulations, and *in silico* biology are all used to interpret experiments, explore the robustness of molecular and mechanical processes, and make predictions.

This review will focus on mediolateral cell intercalation during convergent-extension, what is known about the cell behaviors driving this event, how theoretical models have shaped our understanding of the mechanics of morphogenesis, and what gaps remain.

Observations on convergent-extension

The process of gastrulation in the vertebrate embryo patterns cell identities and moves three primary germ-layers (endoderm, mesoderm, and ectoderm) into their definitive locations (inner-most, middle, and outer-most, respectively). As part of gastrulation the embryo lengthens by a process known as convergent-extension (CE; or alternatively "convergence and

extension"; figure 1). The term CE refers to the bulk movement of prospective dorsal tissues of the embryo as they narrow along the embryo's mediolateral axis (i.e. the left-right axis; figure 1B) and lengthen along the embryo's anterior-posterior axis (sometimes referred to as the rostral-caudal axis). CE brings prospective dorsal tissues from a broad area of the early embryo and organizes them into a compact column that runs from the later stage embryo's head to its tail (figure 1C; see (Keller, 2002)). A variety of cell behaviors such as directed cell migration, mediolateral cell intercalation, radial cell intercalation, asymmetric cell division, cell ingression, and asymmetric multicellular rosette resolution have been proposed to drive bulk CE tissue movements during vertebrate gastrulation (Gong, Mo et al., 2004; Stern, 2004; Solnica-Krezel, 2005; Keller, 2006; Wagstaff, Bellett et al., 2008). Not all of these cell behaviors occur simultaneously but instead sub-sets of behaviors may be used together as adaptations to the physical organization of the pre-gastrula embryo. For instance, early stage amniote embryos, like the chick embryo, take the form of a single epithelial sheet spread over a large yolk mass. In order to move into the embryo individual mesoderm cells constrict apically and leave the epithelium in a process known as ingression (Shook and Keller, 2003). In the case of chicken gastrulation, mesoderm cells appear to intercalate mediolaterally both before and after ingression (Voiculescu, Bertocchini et al., 2007). For the remainder of the review we will focus on mediolateral cell intercalation behaviors driving CE in the frog *Xenopus laevis* and direct readers to papers listed above for details of alternative cellular strategies for driving CE.

Four observed rules that guide cell rearrangement during CE

A minimal description of mediolateral cell intercalation involves oriented cell rearrangement between just three mesenchymal cells in the embryo. One of the cells in the cluster moves between two neighboring cells (intercalating cell marked by asterisk; figure 1C). The moving or intercalating cell separates its two neighbors to form a linear array of three cells. There are at least four key "rules" observed during mediolateral intercalation that may allow intercalation to efficiently drive CE: 1) the intercalating cell moves in a mediolateral direction and separates neighboring cells along the anterior-posterior direction (*planar polarity*; figure 2A), 2) the intercalating cell stays in the same plane as the two neighbors (*remain in-the-plane*; figure 2B), 3) the intercalating cell does not reverse direction and de-intercalate (*irreversibility*; figure 2C), and 4) the intercalating cell and neighboring cells maintain their shapes and do not re-organize within the same volume (*cell shape constraint*; figure 2D). This minimal description of mediolateral intercalation and rules observed by intercalating cells is thought to assure efficient CE (Wilson, Oster et al., 1989; Wilson and Keller, 1991; Shih and Keller, 1992; Shih and Keller, 1992; Domingo and Keller, 1995; Elul, Koehl et al., 1997; Davidson and Keller, 1999; Elul and Keller, 2000; Ezin, Skoglund et al., 2003; Ezin, Skoglund et al., 2006). This basic description and observed rules guiding mediolateral cell intercalation have formed the basis of an ongoing molecular dissection of CE (Keller, 2002).

The molecular basis of two observed rules of cell behaviors, planar-polarity (#1) and remain in-the-plane (#2) have been active topics in cell and developmental biology. Proteins in the non-canonical Wnt or planar cell polarity (PCP) pathways appear to control the mediolateral (ML) and anterior-posterior (AP) orientation of cells during CE in most vertebrates, either directing those cells to intercalate mediolaterally or allowing them to sense other orienting signals (Wallingford, Fraser et al., 2002; Voiculescu, Bertocchini et al., 2007; Yin, Kiskowski et al., 2008). During CE, intercalating cells also remain positioned "in-the-plane" (Keller and Danilchik, 1988; Myers, Sepich et al., 2002; Davidson, Marsden et al., 2006; Keller, Shook et al., 2008; Ninomiya and Winklbauer, 2008) possibly preventing intercalation from driving tissue thickening. Signals from fibronectin (FN) assembled into fibrils or non-fibrillar FN localized at tissue interfaces can provide cues that prevent cells from crawling over or under their neighbors and thus act to keep all three intercalating cells in-the-plane (Davidson,

Marsden et al., 2006; Rozario, Dzamba et al., 2008). Mesoderm adjacent epithelial cells, with apical-basal polarity established by factors such as discs-large (Dlg) and atypical protein kinase C (aPKC) and maintained by cadherin-mediated adhesion, can also provide cues to keep intercalating cells in-the-plane (Ninomiya and Winklbauer, 2008). However, the question of how cells transduce these cues and integrate them with their programs of cell motility and shape change remain to be resolved.

In contrast to the advances on the molecular basis of cell polarity, the basis of the irreversibility rule of cell intercalation (#3) is poorly understood. Mediolateral cell intercalation is not always efficient, for instance, cell intercalation during early mesodermal CE has been termed "promiscuous" or "radical" as cells intercalate freely in both medial and lateral directions, often reversing directions. The same inefficient pattern of intercalation, within lower rates of tissue extension, is exhibited by deep neural cells in explants deprived of underlying mesoderm and accompanying ECM (Elul, Koehl et al., 1997; Elul and Keller, 2000). Neural cells within more complete tissue explants (Elul and Keller, 2000) show "conservative" intercalation by repeatedly intercalating in the same direction or between the same cohort of neighboring cells. The molecular factors mediating persistent intercalation movements or maintaining the cohesion of a cohort of intercalating cells are unknown.

Little is known about the factors that maintain or constrain cell shape as cells intercalate (rule #4). Shih and Keller (1992) made a careful study of cell shape changes in *Xenopus* over the early stages of CE. They found that early gastrula cells are isodiametric then progressively elongate in the mediolateral direction. Cells within mesoderm often lengthen along the mediolateral axis and narrow along the anterior-posterior axis during convergent extension. Elongating cells adopt aspect ratios (ML length to AP width) of 2 to 4 as gastrulation proceeds. From gastrulation through neurulation the embryo elongates in the anterior-posterior direction. Zebrafish mesodermal cells also become elongate but to a lesser degree (length to width ratio of 2) (Concha and Adams, 1998; Myers, Sepich et al., 2002). Paradoxically, as cells continue to intercalate their increasing length to width ratio runs counter to the overall tissue shape change, thus reducing the contribution of mediolateral cell intercalation to CE.

Mechanics and the constraints on cell shape during mediolateral cell intercalation

In the second half of this paper we will survey several efforts to account for cell movements, cell shape changes and mechanics during CE. We then conclude with a semi-quantitative model that relates traction forces and elastic resistance to deformation. This model can account for the progressive cell shape changes of intercalating cells. We hypothesize that intercalating cells gain a mechanical advantage as they elongate.

It is widely acknowledged that cell and tissue mechanics play important roles in shaping the early embryo and that genes control embryonic mechanical properties and regulate cellular force-production driving these movements (Ingber, 2006; Lecuit and Lenne, 2007; Quintin, Gally et al., 2008; Davidson, Von Dassow et al., in press). This view is supported by experimental studies. For instance, identification of important structures can be tested by microsurgically removing a structure or genetically removing precursor cells that give rise to the structure. The role of specific proteins can be tested by eliminating molecular pathways or modulating protein function. The consequences of these experimental manipulations, and the role of a particular structure or protein is revealed by changes in embryo morphology or phenotype.

Unambiguously identifying mechanical processes during morphogenesis can be challenging. When testing the role of a particular pathway that may mediate mechanical processes, a

stringent test requires one to rule out the role of the pathway in overtly altering cell identity. Perturbing protein function in a cell or altering the environment surrounding a cell during development may cause the cell to alter gene expression or even alter the cell's type. Thus, efforts to isolate mechanical processes must also confirm cell identities have not been changed since these changes can change both cell behaviors and the local mechanical microenvironment. For instance, consider molecular or genetic perturbations to the canonical or non-canonical Wnt signaling pathway (Komiya and Habas, 2008). Both canonical and non-canonical pathways share Wnt ligands and frizzled receptors but the canonical pathway regulates cell identities and the non-canonical pathway orients cell behaviors. Thus, cell identities must be confirmed when "mechanical" proteins involved in cell motility or tissue cohesion knocked out or mutated (see reviews (Hutson and Ma, 2008; Davidson, Von Dassow et al., 2009)). Thus, from one perspective we "know" that many proteins are involved in force generation and physical mechanics, however, we would be unable to predict the effect of a single mutation. Extending molecular structure-function studies to the multicellular scale of an embryonic tissue or even a whole embryo is difficult.

Models extend intuition to complex mechanics of morphogenesis

Theoretical models, computer simulations, or *in silico* studies offer the means to explore the complex physical mechanics of morphogenesis and have been used extensively to understand the process of mediolateral cell intercalation. In the section below we will review three examples of models (Weliky, Minsuk et al., 1991; Zajac, Jones et al., 2000; Brodland, 2006) that each capture the outward appearance of mediolateral cell intercalation (figure 3A and B). Each model takes a different approach making both implicit and explicit assumptions of the mechanics and the molecular mechanisms thought to drive tissue morphogenesis.

All the models simulate converging and extending cellular tissues with cell-cell adhesion, cell protrusive or traction forces, and cell rearrangement. The main goals of these models are to make sense of the complex cell intercalation movements and account for the rates of cell rearrangement and cell shape changes that have been quantified during *Xenopus* (Wilson and Keller, 1991; Shih and Keller, 1992) as well as zebrafish (Myers, Sepich et al., 2002) CE. These observations include increases in mediolateral cell elongation – length to width ratio increases from 1.5 at the start of gastrulation to greater than 3 in *Xenopus* and to 2.2 in zebrafish by the start of neurulation – and the incidence of cell-cell neighbor change. Observations also include the rate of cell-neighbor change, i.e. the frequency that a cell contacts a new cell neighbor during intercalation (Shih and Keller, 1992). Each of the three models can account for both changes in length to width ratio and the frequency of neighbor change events even though the implemented rules for cell behavior and assumptions of cellular mechanics differ. In the next section we will review these models and discuss both their explicit and implicit assumptions.

Vertex model

The Weliky and Oster model (1991) we discuss is "agent-based" and simulates a two-dimensional (2D) cellular sheet by a set of individual cells connected to each other along shared boundaries (figure 3C). This model is agent-based since cellular properties are discontinuous and attached to individually modeled cells. This model is also one of the earliest examples of a vertex or network model that represents individual cells in a packed-cell tissue by their connections to neighboring cells (for examples of more recent vertex models see (Farhadifar, Roper et al., 2007) and (Rauzi, Verant et al., 2008)). We refer to this as a Vertex or network model since cell-cell boundaries are represented as a network of segments or short fragments defined by vertices that define the position of the cell boundary. Each cell perimeter is represented by a Newtonian spring with a defined stiffness and rest-length. Other implementations of vertex models have varied the mathematical or physical formulation of the spring (e.g. non-linear) or added forces driven by adhesion-like properties that do not depend

on cell-cell boundary length. In addition to accounting for the cell perimeter, cell volumes must also be simulated. In this model cell volume, or rather 2D area, is maintained around a set value through a pressure term that is applied equally outward at every vertex point on the cell periphery. Pressure is proportional to the deviations between the target cell area and the current cell area, opposing deviations from the target cell area in the manner of a bulk stiffness term.

In addition to the cell volume rule mentioned above several other rules must be implemented to mimic cell behaviors observed in real tissues. For instance, rules are often established to regulate cell volume or area. Consider the case of a real 3D cell modeled in 2D. A cell can change its cross-sectional area in 2D even if it maintains a constant volume in 3D. Thus, special rules are needed to allow or limit cell area changes. To allow changes in perimeter length, segments within the cell-cell boundary can be added or subtracted automatically when individual boundary segments are too extended or too short. Lastly, directional protrusion in the Vertex model is implemented by altering the probability of protrusion at specific vertices in each cell. The probability or frequency of protrusions at a vertex is based on the persistence of previous protrusions, the degree of cell elongation, and contact inhibition. These rules recreate 'feedback' loops thought to reinforce protrusions at the ends of elongating cells and at locations where neighboring cells are moving away. Protrusions are then implemented by 'relaxing' the spring tension at that vertex. Relaxed tension combined with internal outwardly directed pressure allows the cell to protrude.

Cell movement and rearrangement are implemented through a complex set of rules in the Vertex model (see also (Oster and Weliky, 1990; Weliky and Oster, 1990)). After probabilities are calculated and stochastic protrusions assigned to each vertex, the cells move and change shape by summing the forces acting on each vertex point and moving the point according to Newtonian viscous drag, as if the vertex point were an object embedded in a viscous media. Complex rules are also implemented to allow cells to form contacts with new cells or remove contacts with cells that are being left behind. These rules bear some similarity to "transition" rules between surface tension generated cellular structures (Zallen and Zallen, 2004) but are mechanically unrelated since four-cell junctions within a mesenchymal sheet do not appear to be energetically unstable. Both cell shape change and cell rearrangement seen in *Xenopus* CE are successfully modeled within the Vertex model only after cells at the edge of the modeled tissue are flanked by two protrusion-inhibiting notochord-somite boundaries aligned parallel to the AP axis.

Cellular Potts Model

We discuss below the 2D model of convergent extension developed by Zajac, Jones, and Glazier (Zajac, Jones et al., 2003) in which cell-cell boundaries are represented on a much larger fixed grid along with discretized "packets" of cytoplasm delimiting the contents of each cell (figure 3D). The model we discuss is implemented as a Cellular Potts Model (CP model; alternatively known as a Glazier-Graner-Hogeweg model). CP models have been widely adopted to model problems such as cell sorting and rearrangement thought to be driven by differences in cell-cell adhesion or cell-cell contractility (for examples see (Krieg, Arboleda-Estudillo et al., 2008) or (Kafer, Hayashi et al., 2007)). The CP model represents a large number of mesodermal cells packed together on a single fixed grid. The CP model uses a modeling platform that represents individual cells as a set of contiguous blocks within the grid. Each cell is discretized by breaking down the normally continuous shape of the cell into regularly shaped square blocks. Cell-cell interactions are defined by an energy-like function that tallies the relative energy of each segment of cell-cell or cell-media boundary. The CP model is not an explicitly mechanical model since this function does not necessarily represent the physical energy stored in the system. All cell movements and rearrangements are the result of cellular

packets being rearranged to minimize the energy of the field of modeled cells on the regular grid.

Like the Vertex model for CE, the CP model also requires additional rules to maintain cell size and resist cell shape change. The rules that oppose changes in cell size produce a "stiffness-like" property that maintains cell size. The standard formulation of CP models for use with cellular tissues implements an energy term that opposes changes in cell size. Movements of cytoplasmic packets or packets along cell-cell interfaces that increase or decrease cell volume beyond the target volume, or area in the 2D model, are inhibited with an energy penalty while movements that restore a cell to its target size are allowed. Movement of packets that would elongate or change the cell shape from the targeted goal face an energy penalty whereas those that guide the cell shape toward the target shape are allowed. Thus, in the CP model of convergence and extension, cell size and shape changes from pre-defined standards are inhibited and restoring changes are encouraged. Cell elongation appears to be driven by feedback between rules that adjust shape to the pattern of adhesions, and rules that adjust adhesions to the shape. Local cell elongation occurs rapidly in the first few iterations of the model so it appears nearly instantaneous (see figure 5 in Zajac et al., 2003).

Finite Element Model

The last model we discuss is again a 2D model that represents the mechanics of cell rearrangement explicitly within a Finite Element model (FE model; (Brodland, 2006); see (Brodland and Veldhuis, 2006) for parametric analysis of the same model). FE models are widely used in bioengineering and biomechanics and have been adopted for biomechanical analysis of early development (Cheng, 1987; Cheng, 1987; Davidson, Koehl et al., 1995; Brodland, 2006) and organogenesis (Ramasubramanian, Latacha et al., 2006; Cheshire, Kerman et al., 2008). FE models can represent the cells by dividing individual cells into a set of small regular solid shapes such as triangles (see figure 3E). FE models, like CP models, calculate the energy of a structure and deform that structure in such a way as to minimize that energy. Whereas choices to minimize energy in a CP model are made locally, the full shape or global configuration of a structure is optimally deformed in a FE model. Cell volume is explicitly accounted for by a material's Poisson ratio (Vincent, 1990) within an solid FE model. However, in general FE models are used to investigate the passive movements of continuum structures in response to externally applied forces; the FE model designed to account for cell-rearrangement requires significant modifications to basic algorithms (Chen and Brodland, 2008).

The FE model of convergence and extension constructed by Brodland (2006) required two major modifications to the generic FE approach discussed above: 1) contractile rods representing traction forces along the mediolateral cell axis were installed at random locations and triggered stochastically, and 2) special algorithms were developed to track cell-cell boundaries within the FE mesh and oversee the resolution of cell neighbor changes. Both FE and Vertex models must implement special algorithms to allow cell rearrangement. Earlier FE models by Brodland and coworkers (Brodland and Chen, 2000; Chen and Brodland, 2000) detail the algorithms used to re-mesh cells as they rearrange and change neighbors.

These two modifications alone do not allow Brodland's FE model to recreate convergence and extension of *Xenopus* dorsal tissues and require additional input from the mechanical environment (e.g. boundary conditions) of converging and extending tissues. Models with free-moving lateral boundaries, e.g. analogous to a free-floating tissue explant, exhibit many cell-cell rearrangements but do not match progressive cell shape elongation along the cells' mediolateral axis. Alternatively, fixed lateral boundaries, akin to culturing cells on a rigid substrate, reduces the number of cell-cell neighbor changes but then produces elongated cells.

Self-organization and emergent properties from CE models

The basic aspects of CE can be satisfactorily modeled by all three approaches, however, each modeling platform makes different assumptions or rules on cell behaviors and cell mechanics, exhibit different levels of self-organization, and can account for different aspects of CE. The three models established that mediolateral cell intercalation with CE could be driven by: 1) elastically coupled cells (Vertex model), 2) differential adhesion (CP model), or 3) mediolaterally directed traction forces (FE model). An important but underappreciated point is that in order to work, each of the models incorporate some approximation of cell stiffness.

Planar polarity—Both the Vertex and CP models (but not the FE model) appear to produce planar polarity (as one of the four rules defined above) as an outcome of local feedback rules without any initial bias (e.g. an emergent property). In the Vertex model emergent polarity depends on having boundaries that inhibit protrusions, but this matches what is known from experimental observations (Shih and Keller, 1992; Domingo and Keller, 1995) so does not seem to count as "pre-conditioning" in any meaningful sense. The CP model does not appear to include any rules to create a globally preferred orientation for cell-cell adhesion or to drive elongation. Elongation and orientation appear to be emergent properties of the feedback rules, just as they are in the Vertex model (this was not true in the FE model where the bias was built in).

Remain-in-plane—None of these models simulates more than two dimensions so cell movements are "pre-conditioned" to remain-in-plane.

Irreversibility—It is arguable that the Vertex model does produce irreversibility as an outcome from local persistence at the level of vertices or from contact inhibition; the other two models do not clearly produce it as an outcome.

Cell shape constraint—Cell shape/size constraints are not imposed in the vertex or FE models, except as an outcome of the assumed balloon-like (Vertex model) or bubble-like (FE model) cell mechanics. The cell shape and size constraints of the CP model seem to be analogous to a crude approximation to shear and bulk stiffness.

Cell and tissue mechanics are essential for emergence of CE in all the models. All three models, especially CP, make a good case that cell stiffness, hence tissue stiffness, is a pre-requisite for correct CE. Furthermore, the emergence of CE from local cell rules in both the Vertex model and the FE model are explicitly dependent on specific boundary conditions.

The "Wedge": a simple semi-quantitative model suggested by complex rule-based models

Cell shape change emerges independently from all three models of CE and suggests a minimal mechanical model for cell intercalation based on the simple wedge. We can consider the static mechanics of a cell intercalating between its anterior and posterior neighbors as if the intercalating cell was a wedge (red triangle; figure 4A) being driven between its two neighbors (green blocks; figure 4A). We can write an equation describing the forces needed to initiate intercalation. Intercalation begins when traction forces (F_{traction}) pushing or pulling the cell overcome friction (μ_s) and the elastic resistance of the neighboring cells ($F_{\text{elastic resistance}}$):

$$F_{\text{traction}} > F_{\text{elastic resistance}} (\mu_s + \mu_s \cos \alpha + \sin \alpha) \quad (1)$$

The length (L) and width (W) of the intercalating cell dictate the intercalation angle, α , of the wedge ($\alpha = \arctan (W/L)$).

We can draw several conclusions from the wedge-model without the need of a more complex simulation. First, the forces required for intercalation are reduced for a more elongate cell whether friction is high or low (figure 4B). Second, if we assume the cell and its environment are made of elastic materials, this simple model also predicts that cells will progressively elongate as they intercalate. As the intercalating cell 'wedges' between its neighbors they will be compressed along with the intercalating cell (figure 4C). Even the least amount of resistance to elongation at the anterior and posterior ends of the block (i.e. boundary conditions) will compress the cell in the AP direction (ΔL ; figure 4C). Strain in response to wedging, resistance of the material to change volume (e.g. the Poisson ratio), and compression along the AP direction then elongates the cell in the ML direction. Lastly, these conclusions do not rely on the specific shape of the wedge but are general properties of the wedge-model. Thus, a simple wedge model suggests feedback between intercalation, reduction in forces required for intercalation, and cell elongation.

The wedge-model is an example of a simple machine that can reproduce mediolateral cell elongation while taking into account cell shape and mechanical properties such as tissue stiffness and boundary conditions. Given the growing complexity of multicellular models, we hope efforts by modelers and theorists will reveal universal mechanical principles that allow the broader fields of cell and developmental biology to understand the complexities of morphogenesis.

Future Directions

Successful models of CE need to produce more than the right shape of cells and tissues, they must produce the right shapes under conditions of stiffness and force production observed in real embryos (von Dassow and Davidson 2009; Zhou et al 2009; Kalantarian et al 2009). Despite rapid recent advances, such mechanical realism has not yet been checked by any of these models. Additionally, the three models we've reviewed suggest a series of experimental tests: Do notochord cells elongate autonomously when dissociated or transplanted to other sites in the embryo at approximately the right time, or do they require (as predicted by FE and vertex) unique boundary conditions provided by surrounding tissues? Furthermore, does cell elongation vary between embryo, free explants, or explants cultured on glass? Does the length to width ratio of cells match what one would expect based on the FE and Vertex models with their required boundary conditions?

The CP and Vertex models successfully generate polarity but future models should simulate the conditions in a more transparent, biologically relevant manner. One of the next challenges for CE models will be to integrate subcellular signaling networks such as the non-canonical Wnt pathway (e.g. modeled in multicellular epithelia (Amonlirdviman, Khare et al., 2005)). Another challenge for future models of CE will be to incorporate fine details of cytoskeletal regulation such as the Rho family GTPases (modeled in single cells (Mori, Jilkin et al., 2008)) and include mechanically realistic tissues of the embryo (von Dassow and Davidson, 2009; Zhou, Kim et al., 2009).

In order to test more complex mechanisms that self-organize planar polarity without boundary conditions, remain-in-plane, irreversibility, and cell shape constraint, future models will need to (respectively): 1) autonomously generate subcellular polarization that can direct mediolateral cell protrusions, 2) represent multiple cell layers or even full 3D cells, 3) autonomously generate persistent cell intercalation behaviors, and 4) represent more realistic material properties of both intercalating cells and the tissues they form. Furthermore, new models will need flexibility so they may include specialized adaptations to CE within different organisms such as mouse, chick, and zebrafish and during the formation of organs such as the heart, kidney, and bone.

However, the vastly increasing complexity of models highlights the need for greatly simplified models, such as the wedge model, that can suggest general properties revealed by complex models, and aid in understanding the results of those complex models.

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Figure 1. Gastrulation, elongation, convergent extension, and mediolateral cell intercalation

A) Gastrulation is shown as a topological process that converts a ball into a toroid and then elongates the toroid into a tube. B) Gastrulation and elongation produce a tadpole, analogous to a tube, from a ball of cells. C) Convergent extension (CE) of dorsal tissues (red) in the embryo is the result of cell rearrangement in the tissue. Cells (drawn as ellipses) intercalate between their mediolaterally (ml) adjacent neighbors driving them apart along the anterior-(a)-posterior-(p) axis. Several rounds of mediolateral cell intercalation drive convergent extension and elongate the embryo.

Figure 2. Observed 'rules' of cell intercalation

Four rules of cell intercalation have been observed during CE movements. A) *Planar polarity* involves the progressive orientation of non-polarized cells. Cells must first localize factors at mediolateral surfaces (m/l; asterisks) or alternatively their anterior-posterior surfaces (not shown). Polarized factors then manifest by polarized protrusions and polarized cell shape change in intercalating cells (right-most panel). B) The *remain-in-plane* rule increases the efficiency of CE by keeping all three cells within the same plane. Converging cells crawling on top of or below their neighbors would reduce the efficiency of CE by thickening rather than elongating the tissue. C) The *irreversibility* rule stems from the need to lock in mechanical "gains" of intercalation. If the direction of cell intercalation is reversed in the next round the

contribution to elongation of the tissue is lost. D) The *cell shape constraint* is also needed to "lock in" the mechanical gains of elongation. If cells merely change shape after intercalation, accommodating to their former space (grey rectangle), there will be no CE and no change in overall tissue shape.

Figure 3. Cell intercalation within a multicellular tissue and three models of intercalation

A) An image from a confocal time-lapse sequence collected 5 μm from the adhesive surface shows the extent of cell bodies (numbered 1, 2, and 3) expressing a membrane-localizing GFP. B) The same field of cells one hour later has rearranged. Cell 2 has intercalated between cell 1 and cell 3. The anterior-posterior axis runs top-to-bottom and the mediolateral axis runs left-to-right. C) A Vertex model captures the geometry of cell outlines for the three cells in a series of segments (one segment between each dot). D) A Cellular Potts model captures the three cells within a fixed grid. Contents (different shades) of the three cells and grid-elements along interfaces contribute to the overall energy of the configuration. E) A Finite Element model captures the tensile boundaries and viscous contents of the three cells in a set of triangles. The

outlines for each of the three cells in panel A have been used as a source of the schematic in panels C, D and E. The scale is the same for panels A and B.

Figure 4. Modeling cell intercalation with the wedge, a "simple" machine

A) Schematic of a wedge with a defined intercalation angle α (red; dark grey) statically sitting between two elastic blocks (green; light grey). A polarized traction force pushes the wedge between the elastic blocks. B) Plot of the threshold for movement $F_{\text{traction}} / F_{\text{elastic resistance}} > (\mu_s + \mu_s \cos \alpha + \sin \alpha)$ for a range of friction coefficients as cells elongate. Cells with a particular length to width ratio are shown as both ellipses and their equivalent wedge. The wedge angle α is the angle subtended by mediolateral "corner" of the cell. C) Once traction forces on the wedge overcomes friction and elastic resistance the wedge moves between the elastic blocks pushing them apart a distance ΔL . If elongation is completely resisted the elastic blocks and wedge are both compressed and the wedge angle narrows from α to α' .