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Biology and Physics of Cell Shape Changes in Development

Review

Ewa Paluch[1,2] and Carl-Philipp Heisenberg[1]

Together with cell growth, division and death, changes in cell shape are of central importance for tissue morphogenesis during development. Cell shape is the product of a cell's material and active properties balanced by external forces. Control of cell shape, therefore, relies on both tight regulation of intracellular mechanics and the cell's physical interaction with its environment. In this review, we first discuss the biological and physical mechanisms of cell shape control. We next examine a number of developmental processes in which cell shape change — either individually or in a coordinated manner — drives embryonic morphogenesis and discuss how cell shape is controlled in these processes. Finally, we emphasize that cell shape control during tissue morphogenesis can only be fully understood by using a combination of cellular, molecular, developmental and biophysical approaches.

Introduction

How tissues and organs are shaped during embryogenesis is a fascinating and long-standing question in developmental biology [1]. As the cell is the functional unit of any living tissue, all shape changes in the organism are driven by events at the cellular level. In combination with cell division, growth and death, changes in individual cell shape are central to morphogenesis. Here we focus on how cell shape is controlled and functions during the shaping of embryonic tissues.

The shape of the cell is defined by the geometrical information of the space occupied by the cell and is determined by its external boundaries. It is the result of the mechanical balance of the forces exerted on the cell membrane by intracellular components and the outside environment [2,3]. As such, it is essentially a physical property controlled by a variety of biochemical pathways. Therefore, to study how cells control their shape, we need to understand how they regulate their own mechanical properties.

Intrinsic forces exerted on the membrane are mostly the direct result of the reorganization of the cytoskeleton. Cytoskeletal networks can, for example, contract the cell during cytokinesis [4], migration [5–7], and apical contraction [8]. Polymerization of actin networks can also drive membrane extension and formation of protrusions such as lamellipodia and filopodia [9,10]. Finally, intracellular pressure of osmotic origin or generated by contraction of cytoskeletal networks can lead to cell deformations [3,11,12]. Extrinsic forces exerted on the cell are mainly due to its adhesion to environmental components, such as neighboring cells or the extra-cellular matrix (ECM). The resulting balance of intrinsic and extrinsic forces needs to be tightly regulated in order to ensure controlled cell shape changes. Such regulation requires constant feedback between cellular mechanical properties and gene expression/protein activation.

The biomechanics of cell shape have so far been mainly investigated in individual cells in culture [3,13–16]. Shape change of individual cells, independent of their neighbors, contributes to different morphogenetic processes in development, such as the migration of single primordial germ cells towards the gonad [17,18]. However, in most morphogenetic events, cell shape change is coordinated amongst hundreds of neighboring cells and drives shrinkage, extension, folding and movement of tissues.

Here, we first summarize the current knowledge about how the mechanical properties of cells are regulated and how they contribute to cell shape change. We particularly discuss the importance of mechanosensing for the regulation of cellular mechanics. We follow this by examining examples of developmental processes in which cell shape change has been studied. We particularly focus on how regulation of a cell's mechanical properties is achieved *in vivo* and on how shape change at the single cell level is coordinated among neighboring cells to achieve global tissue shaping.

Mechanical Properties of Cells Govern Cell Shape

Biochemical and genetic studies have long dominated the field of morphogenesis and have yielded a wealth of information as to how patterns and shape change are controlled during development [19,20]. However, the shape of cells and tissues is a mechanical issue, directly relating to the balance of physical forces exerted on the cell surface [1,2,21,22]. Therefore, to unveil the mechanisms of shape control we need to understand how the cell regulates its mechanical properties and the forces it generates.

Forces exerted on the cell surface can be subdivided into three main categories: forces actively generated within the cell, e.g. by polymerization or contraction of cytoskeletal networks, or by opening of water or ion channels leading to changes in osmotic pressure; forces exerted on the cell from the outside, either directly by neighboring cells or indirectly through the ECM; forces generated within the plasma membrane itself by, e.g., lipid segregation or recruitment of curvature-inducing proteins. Here, we will focus entirely on the first two categories, as they are likely to constitute the main factors in shape change on the scale of the entire cell.

A major limitation of mechanical studies of cell shape is the difficulty in directly measuring forces exerted on the plasma membrane. Cellular mechanics are thus usually described by a set of physical properties that can be inferred from measurements. These include cell adhesion to its environment, cortical tension and cell material properties such as stiffness or viscosity (Figure 1). Although many methods have been developed to measure these properties in isolated cells (Box 1), most of them are not directly transferable to cells inside tissues and embryos. Therefore, studies of morphogenesis in many cases must still rely on data obtained from measurements of dissociated cells in culture. Notable exceptions from this are recent studies that used

[1]Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany. [2]International Institute of Molecular and Cell Biology, 4 Ks. Trojdena Street, 02-109 Warsaw, Poland.
E-mail: paluch@mpi-cbg.de (E.P.), heisenberg@mpi-cbg.de (C.-P.H.)

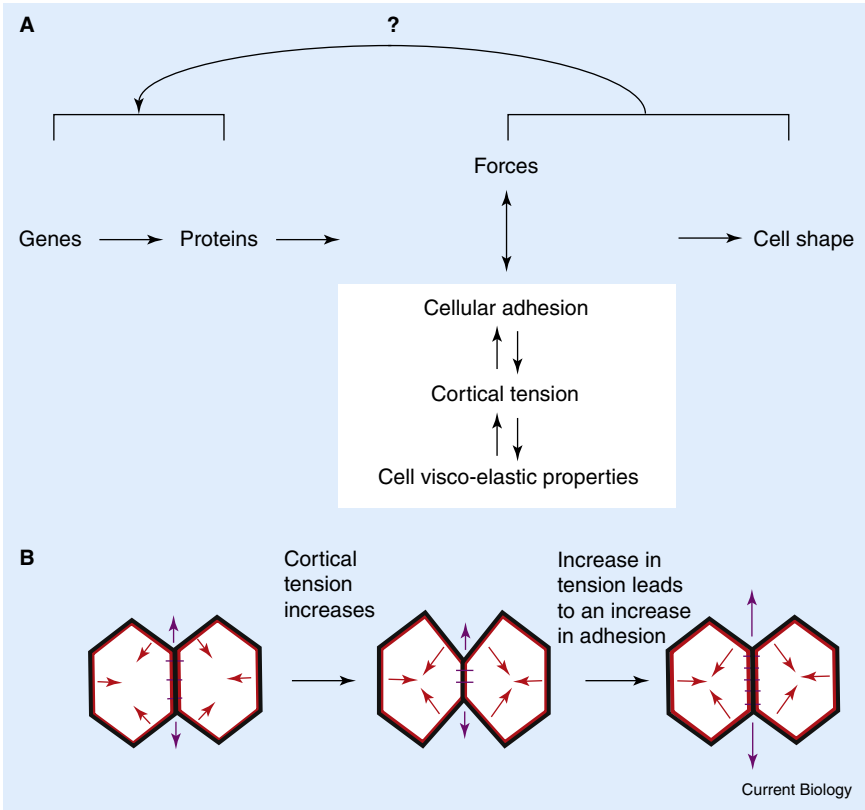


Figure 1. Regulation of cell shape.

(A) Cell shape is the result of the mechanical forces exerted on the cell surface. These forces can also be described by a set of interrelated physical properties, which include cellular adhesion, cortical tension and the cell's rheological properties. Physical properties are regulated at the protein level and are likely to feedback on protein activity and/or gene expression. (B) Example of the interaction between cortical tension and cellular adhesion during the formation of cell–cell contacts. Red lines, cortical cytoskeleton; black lines, plasma membrane; purple rods, adhesion sites; arrows, direction of forces.

methods such as laser ablation to measure tension at the boundaries between cells or tissues in living embryos [23-26]. Laser ablation can also provide a means to estimate the viscoelastic properties of the ablated cell or interface [23,24], although most rheology measurements *in vivo* have so far been performed at the level of the whole embryo or on isolated tissues [27]. In contrast, there are currently no reliable assays available to measure cell–cell or cell–substrate adhesion *in vivo*. The development of accurate methods to measure the mechanical properties of individual cells directly inside tissues or embryos is essential to move our understanding of the mechanics of morphogenesis beyond a descriptive level.

Because the cell is a complex object, the interpretation of any quantitative experiment relies on a physical model describing how a mechanical property of interest can be deduced from the actual measurement. It is, therefore, essential to test the premises of the model experimentally. For example, in micropipette aspiration experiments (Box 1), the formula used to relate the pressure inside the pipette to cortical tension depends on whether the cell behaves like a liquid drop surrounded by a contractile shell or like an elastic solid [28–30]. Similarly, in compression or atomic force microscopy indentation experiments, one must make assumptions about how the cell behaves under load to infer cortical tension from force-deformation measurements. Here again, most models describe the cell as a viscous drop surrounded by a contractile cortical shell; however, this relies on a number of assumptions that need to be verified independently [31,32]. In situations, in which the cell is substantially deformed during the measurement, such models can prove insufficient, and the nucleus, which is considerably stiffer than the cytoplasm, has to be included in the description [33,34].

Moreover, because the cell is an active object, it can react to mechanical perturbations. It has been shown, for example, that mitotic *Dictyostelium* cells recruit myosin II to the cortex upon micropipette aspiration and retract from the pipette [14], making tension measurement by micropipette aspiration impossible (Box 1). Similarly, fibroblasts reinforce focal adhesions upon pulling [35], complicating the interpretation of classical adhesion measurements (Box 1). Such active behaviors limit the use of traditional physical techniques for the measurement of cellular mechanical properties, and emphasize the need for developing less-invasive methods.

It is important to note that cortical tension, cellular adhesion and cellular viscoelastic properties are not independent but are mechanically coupled to each other (Figure 1). The global viscoelastic properties of a cell depend on its cortical tension as well as on the viscoelasticity of the cytoplasm [29,31]. Furthermore, cortical tension directly influences cell–cell adhesion. For two adhering cells, a uniformly high tension will tend to favor a spherical cell shape and thus reduce the contact area between the cells, which again, for a given concentration of adhesion molecules, will diminish the adhesion force between the cells [22,36]. Similarly, in epithelia with actin-myosin bundles at adherens junctions, the length of cell boundaries is reduced by a high degree of cell–cell adhesion and increased by high bundle contractility [23] (Figure 1B). In addition to being mechanically coupled, the different mechanical properties are often regulated by overlapping biochemical pathways. For example, proteins regulating actin turnover are likely to influence cortical tension as well as the cell's viscoelastic properties [37]. Also, E-cadherin expression has been shown to strengthen membrane–cortex attachment, which again can affect cortical tension [38]. The various mechanical properties of a cell are thus connected both genetically and mechanically. It is, therefore, extremely difficult to study them independently of each other and to separate their respective influence on cell shape.

Given the multitude of factors that can influence a given mechanical property of a cell, its precise control represents an enormous challenge to each cell. Strikingly, in spite of the complex regulation and caveats linked to experimental

measurements, mechanical properties often appear well-defined for a specific cell type while varying considerably between different cell types, at least when measured on isolated cells. Cortical tension and cell–cell adhesion, for example, are significantly different between the germ layer progenitor cell types in zebrafish, and these differences can account for segregation of cells into tissues [32]. How these properties are controlled is still an open question, but it is likely that cells use a set of feedback mechanisms to set and maintain specific mechanical properties.

At a more global level, cells may also sense and directly control their own shape. Several examples of mechanosensing feedback on the organization of adhesion sites and the cytoskeleton have been reported in culture cells. Mitotic *Dictyostelium* cells, for example, actively respond to deformation by locally recruiting myosin II to counteract the shape change, suggesting a sensing and active control of cellular shape [14]. Global mechanosensing pathways could allow cells to compensate for the change in one mechanical parameter by adjusting another. Increasing tension in cultured fibroblasts, for example, can increase the strength of focal adhesions [35,39]. Similarly in endothelial cells, cellular stiffness increases with increasing adhesion to the substrate [40]. It is likely that such cross-regulation is also employed by cells in tissues and embryos, although this has not yet been directly shown. In epithelia, for example, where adhesion and cortical tension have opposing effects on the interfacial tension at cell–cell boundaries [22,36], a positive feedback loop between adhesion strength and cortical tension could help stabilize the length of the boundaries (Figure 1B). Further work will be necessary to elucidate which mechanical properties are controlled by mechanosensing pathways during development. Estimating the level of variability of cellular mechanical properties measured inside tissues and embryos may help in answering this question, as the presence of a feedback loop is likely to reduce variation.

Various models have been developed to describe how the interaction between cellular mechanical properties governs the shape of the cell and the arrangement of cells into aggregates or tissues [22,41]. Seminal studies by Steinberg [42,43] proposed that similar to liquid droplets, cell sorting in tissues and aggregates is driven by interfacial tension. Although interfacial tension between cells was initially thought to be predominantly determined by cell–cell adhesiveness [44], a number of recent experiments indicate that cortical tension may also play an important role in cell sorting and organization into tissues [23,32]. Moreover, although cell stiffness and viscosity have only been little studied *in vivo*, the control of these material properties is also likely to be essential during morphogenetic movements, as cellular viscoelasticity determines how cells deform in response to internal and external forces [23,37]. It will be important in the future to determine how cell–cell adhesion, cortical tension and cellular material properties are fine-tuned to drive specific tissue deformation [21].

Cell Shape Changes in Development

To describe developmental processes in which cell shape changes are critically involved, one faces two major questions: Which morphogenetic processes in the embryo are directly driven by changes in cell shape? Have particular embryological processes been sufficiently analyzed to propose a mechanistic model explaining the contribution of cell shape change?

In order to answer the first question, one needs to distinguish between a number of different basic cell biological processes that have been associated with cell shape change. These processes include cell polarization, cell protrusion formation/migration, cell division, apoptosis and junctional remodeling. Junctional remodeling has been predominantly described in cellular rearrangements underlying epithelial morphogenesis, such as cell intercalation during germ band extension in *Drosophila* gastrulation [45,46]. However, while the molecular and cellular mechanisms underlying changes in the junctional interfaces to neighboring cells have been intensively studied in this process, the contribution of cell shape change as such has not yet been systematically addressed. Similarly, while cell divisions are usually accompanied by considerable changes in cell shape, the specific contribution of these cell shape changes to tissue and/or embryo morphogenesis is still largely unclear [47–49]. In contrast, the two remaining processes, cell protrusion formation and cell polarization, have been closely associated with distinct cell shape changes and implicated in various morphogenetic processes such as cell migration, cell intercalation and tissue invagination. In the following, we will therefore exclusively focus on the role of individual cell shape changes resulting from alterations of cell polarization and protrusion formation during tissue and embryo morphogenesis.

Which developmental process involving cell polarization and/or protrusion formation has been sufficiently analyzed to propose a mechanistic model for the role of individual cell shape changes? As outlined above, in order to fully understand how cell shape change drives tissue morphogenesis, one needs to investigate how specific cellular and physical properties, such as cell adhesion and contractility, are determined biochemically, how those properties influence cell shape, and how coordinated cell shape changes control tissue or embryo morphogenesis. Hardly any information on the physical properties of individual cells in tissues and/or embryos is available and therefore the contribution of these properties to cell shape determination *in vivo* is still poorly understood. Similarly, quantitative analysis of individual cell shape in three dimensions over time in embryos has only just begun and thus insight into the contribution of cell shape change to tissue morphogenesis is still sparse. For the time being, we are therefore left primarily with information about molecular function in global morphogenesis. There are a few morphogenetic processes in development, however, where molecular information on morphogenesis has recently been paired with novel insights into individual cell properties and/or shape.

Below, we will provide an overview of the contribution of cell shape changes to cell migration and tissue morphogenesis in development and of the mechanical processes by which these cell shape changes are achieved and coordinated amongst multiple cells. We will start by summarizing current knowledge on cell mechanics in the fields of single cell migration and collective migration, and then describe recent findings made in three specific developmental processes — dorsal closure and ventral furrow formation in *Drosophila* and convergent extension movements during *Xenopus* gastrulation.

Single Cell Migration in Development

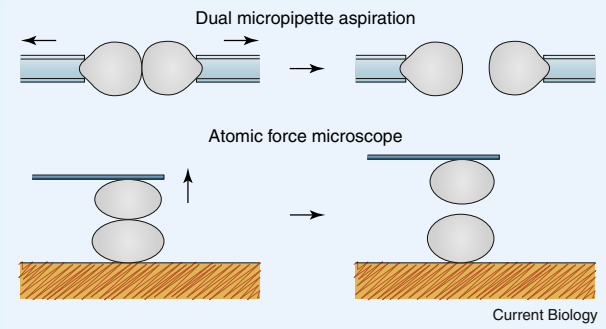
In the development of multicellular organisms, different cell types, such as primordial germ cells and leukocytes,

Box 1

Methods for measuring the mechanical properties of single cells.

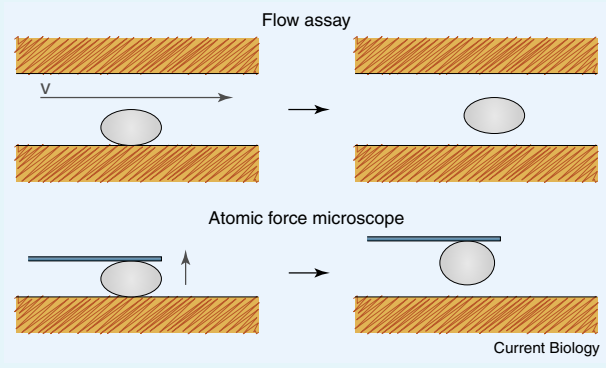
Cell–cell adhesion

Various assays have been developed to measure the force of adhesion between two cells (reviewed in [106]). Many of them utilize micropipettes to hold the cells; the pipettes are then pulled apart until the cells are separated. The force of adhesion, i.e. the force necessary to part the cells, can be measured, e.g. using a red blood cell as a force probe [107], or directly from the pressure in the micropipettes (dual micropipette aspiration) [108,109]. More recently, cell–cell adhesion has also been measured using an atomic force microscope. In this setup, one cell is attached to the cantilever while the second cell is attached to a rigid substrate and the separation force is directly deduced from the deflection of the cantilever [32,110].



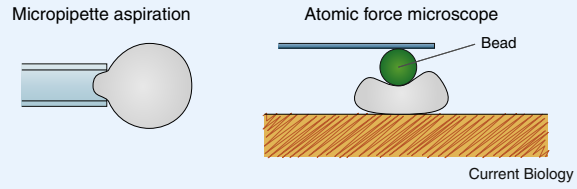
Cell–substrate adhesion

The adhesion of a cell to a substrate has been first assessed in washing assays where a cell is detached from the substrate by a convective flow (flow assay) [106,111]. However, as shear forces exerted by the fluid in a flow chamber depend on factors such as cell geometry, flow chambers do not provide a very precise force measurement [106]. The adhesion of a cell to a substrate can also be measured using an atomic force microscope [112] or optical tweezers [113].



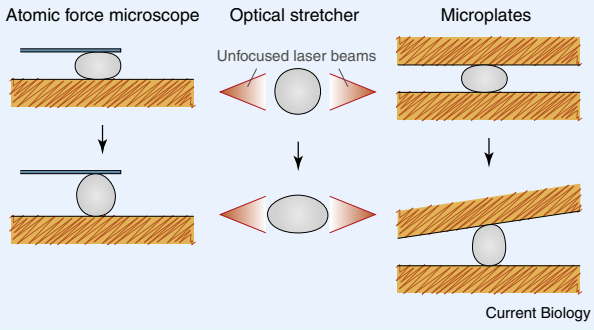
Cortical tension

Most cortical tension measurement methods rely on the assumption that the cell behaves like a liquid droplet surrounded by a contractile cortex. The contractility of the cortex effectively results in a surface tension, which can be deduced from the cell's deformation in response to a controlled force. Typical methods include micropipette aspiration [29,114] and a variety of setups allowing a controlled indentation of the cell (e.g. atomic force microscopy) [31,32]. In some cases, cortical tension can be deducted from micropipette aspiration experiments also when the cell does not behave like a liquid droplet, but a different model must then be used [29].



Cell stiffness and viscosity

A variety of methods have been developed to measure the global viscoelastic properties of single cells in culture (reviewed in [37]). Most of these methods consist in stretching or compressing the cell with a controlled force and monitoring the dynamics of the resulting deformation. Typical experimental setups include atomic force microscopy [115], precisely controlled microplates [116], micropipettes [31] or an 'optical stretcher' where the cell is stretched between two unfocused laser beams [117]. The dynamics of cell aspiration into a micropipette can also provide an estimation of the cell's viscoelastic properties [29,114].



undergo single cell migration. Commonly, single cell migration involves the formation of cellular protrusions at the leading edge and contraction of the trailing edge, resulting in a cell shape that is polarized in the direction of migration [50]. The types of cellular protrusions formed at the leading edge vary considerably between different cell types undergoing single cell migration [51]. For example, primordial germ cells in zebrafish migrate by forming bleb-like protrusions at their leading edge [17] (Figure 2A), while leukocytes in mice form thick actin-rich pseudopodia-like protrusions [6].

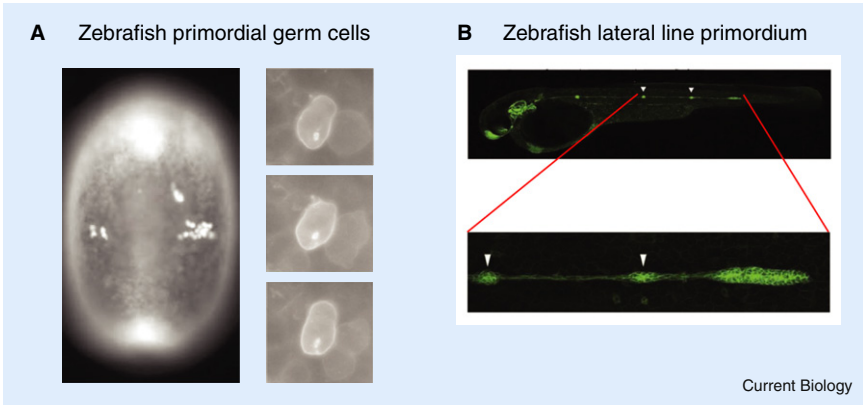


Figure 2. Two examples of developmental processes involving single cell migration and collective migration, respectively.

(A) Migration of primordial germ cells during zebrafish gastrulation. Dorsal view of a zebrafish embryo at bud stage (10 hours post-fertilization) with germ cells migrating from both sides towards the embryonic axis (left panel, anterior to the top). High magnification view of individual primordial germ cells migrating individually (right panels). (B) Migration of the lateral line primordium during zebrafish embryogenesis. Lateral line primordium in an embryo at 42 hours post-fertilization (low and high magnification views in upper and lower panel, respectively; anterior is to the left; arrows point at forming neuromasts). Pictures are adapted from [102–104].

What determines cell shape change in single cell migration? Generally, for a cell to migrate it needs to extend protrusions in the direction of migration, adhere to its substrate, and pull/ squeeze itself forward. Adhesion is thought to function in this process by providing sufficient traction required for efficient translocation [52–54]. In two-dimensional cell culture assays, it has been suggested that there is a precise amount of cell–substrate adhesion needed for optimal cell migration [55]. However, what level of cell–substrate adhesion is required for three-dimensional migration in a developing organism is still unclear. Primordial germ cell motility, for example, has been suggested to rely on at least some level of cell–cell adhesion mediated by cadherins (E. Raz, personal communication). In contrast, leukocytes are able to migrate in the absence of any Integrin-mediated cell–substrate adhesion, and it has been hypothesized that a minimal level of unspecific adhesion might already be sufficient for their migration [6].

Similar to adhesion, different roles of tension for cell shape change in single cell migration have been suggested. At the leading edge, tension exerted by protrusions adhering to a substrate will pull the cells forward, while at the trailing end cortical tension will push or squeeze to cytoplasm in the direction of migration [52]. Consistent with such roles, myosin-dependent contraction at the trailing edge in leukocytes has been suggested to facilitate migration by squeezing the cytoplasm and nucleus forward when the cell migrates through a constricted environment [6]. Moreover, myosin-mediated contraction at the trailing edge has been associated with nucleokinesis during tangential migration of cortical neurons in mice [56]. Finally, myosin-dependent cortical contraction at the leading edge of zebrafish primordial germ cells has been proposed to function in migration by triggering bleb formation through the regulation of local intracellular pressure and/or breakage of the actin cortex [17].

Although the molecular mechanisms underlying single cell migration in two-dimensional cultures have been intensively studied over the past decades [57], it is not known if the same mechanisms are at play during single cell migration *in vivo*. A gradient of extracellular chemokines is thought to guide both leukocytes and vertebrate primordial germ cells. In zebrafish, the chemokine SDF-1 has been proposed to locally increase intracellular Ca^{2+} at the leading edge of primordial germ cells, which in turn activates myosin, cortical contraction, and bleb formation [17]. In flies, germ cell migration is directed by a lipid-modified peptide, the identity of which is still debated [18]. It has further been shown that activation of the G-protein coupled receptor Tre1 in *Drosophila* germ cells triggers cell motility and invasion by down-regulating E-cadherin and redistributing the junctional proteins from the cell periphery to the lagging tail of germ cells [58], pointing to a critical role of spatial regulation of cell adhesion in initiating cell motility.

Collective Cell Migration

Collective cell migration constitutes one major variant of cell translocation in development. There are multiple instances in development where cells migrate collectively, including the migration of germ layer progenitors in vertebrates, lateral line primordial cells in zebrafish (Figure 2B), and border cells in *Drosophila* [21,59]. Common to all these processes is that cells migrate while remaining in contact with each other. Prominent cell shape changes during collective migration are observed at the leading edges of a migrating cell cluster where cells form various types of cellular protrusions and become polarized in the direction of migration. In contrast, cells behind the leading edge are surrounded by other cells, to which they are connected through cell–cell adhesions, and often form fewer or no protrusions.

How is cell shape regulated during collective cell migration? Generally, for cells to migrate collectively, they need to adhere to each other, form protrusions at the leading edges of the cluster and suppress protrusions at cell–cell contact sites. In the absence of proper cell–cell adhesion, cells are unable to form a coherent cluster and thus cannot migrate collectively. When E-cadherin expression is reduced in *Drosophila* border cells, for example, they fail to organize into an epithelial cluster, to orient their cellular protrusions and to migrate towards the oocyte [60–62]. Similarly, interfering with cadherin activity in mesoderm progenitors in *Xenopus* and zebrafish causes defects in germ layer progenitor cell polarization, migration, intercalation and separation [63–68]. In addition to cell–cell adhesion, cell–substrate adhesion is also involved in collective migration. Studies in *Xenopus*, for example, have shown that integrin–fibronectin binding is required for mesoderm polarization and migration, although it is not yet entirely clear how much of this function is mediated by integrins regulating cadherin-mediated cell–cell adhesion [69–71].

The role of tension and contractility in collective migration has only started to be analyzed in development. In *Drosophila*, border cell tension has been suggested to control the nuclear localization of the transcription factor serum response factor (SRF), whose activity is required for

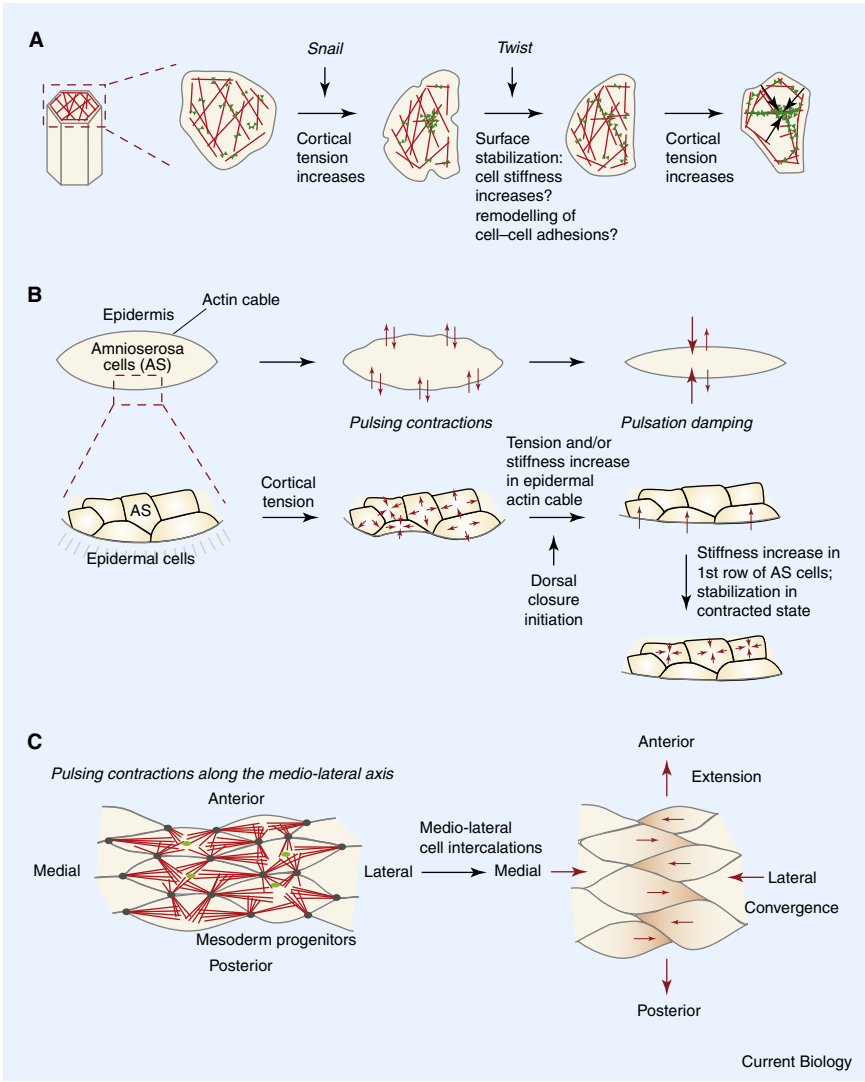


Figure 3. Examples of how sequential modification of cellular mechanical properties drives tissue deformations.

(A) A recent study has shown that ventral furrow formation in *Drosophila* embryos is driven by pulsed apical contractions of mesoderm progenitors [8]. Each cell undergoes a series of cortical contractions, dependent on the transcription factor Snail, separated by pauses during which the cell shape is mechanically stabilized. This stabilization requires the activity of the transcription factor Twist. Because the cell does not relax during the pauses, the pulses lead to global tissue contraction. Figure adapted from [105]. (B) Dorsal closure in *Drosophila*. The closure of the dorsal hole (gray shape) is driven by contractions of a supracellular actin cable and apical constrictions of amnioserosa cells. A recent study has shown that the amnioserosa cells undergo pulsed contractions and that their contractions are stabilized and dampened by the stiffness of the supracellular actin cable within the epidermis [94]. (C) Medio-lateral cell intercalations in *Xenopus* gastrulation. Medio-lateral intercalations of mesoderm progenitors are driven by pulsed contractions of a cortical actin network along the mediolateral extent of these cells [97].

proper migration [72]. Interestingly, SRF was found to function in border cells by building a robust actin cytoskeleton, which is likely to generate higher tension in these cells, thereby providing a positive feedback loop regulating its own activity. A role for tissue tension has also been suggested in migration of mesoderm progenitors during *Xenopus* gastrulation, where the cells exert traction stress on the extracellular matrix, thereby influencing matrix organization required for proper mesoderm migration and polarization [70].

The molecular regulation of collective cell migration in development has been intensively studied over the last years. In the zebrafish lateral line primordium, an extracellular chemokine gradient directs the migration of leading edge cells, which in turn control through Wnt/β-catenin and FGF signaling the epithelial character of the cells following behind [73–75]. Likewise, a gradient of extracellular PDGF and/or VEGF signaling has been hypothesized to control the collective migration of border cells in *Drosophila* [76,77], and of mesoderm progenitors in zebrafish, *Xenopus* and chicken gastrulation [78–80]. Wnt signaling, and in particular the Wnt-Frizzled/planar cell polarity (Wnt-Fz/PCP) pathway, has also been shown to be involved in collective migration [81]. In both vertebrate gastrulation [82] and *Drosophila* border cell migration [83], Wnt-Fz/PCP signaling is essential for cell polarization, directed migration, and intercalation. While it is still largely unclear how Wnt-Fz/PCP functions in collective migration, regulation of actomyosin contraction and cell adhesion has been suggested [84–87]. Another signaling pathway with a conserved function in collective cell migration is the JAK–STAT pathway. In both *Drosophila* border cell and vertebrate mesoderm progenitor cell migration [88–90], JAK–STAT signaling is critical for cells to organize into a coherent cluster and migrate collectively. How JAK–STAT functions in this process is not yet entirely clear, but is has been suggested that STAT3 cell-non-autonomously controls Wnt-Fz/PCP signaling in zebrafish mesoderm progenitors [91].

Ventral Furrow Formation

Ventral furrow formation during *Drosophila* gastrulation is initiated by the coordinated apical constriction of mesoderm progenitor cells located at the ventral-most side of the embryo in a narrow band of cells extending along the anterior-posterior embryonic axis [92]. This process is triggered by the transcription factors *twist* and *snail.* Two transcriptional targets of *twist*, the secreted protein Folded Gastrulation and the transmembrane protein T48, are thought to facilitate apical constriction of ventral furrow cells by translocating RhoGEF2 and, as a consequence, myosin to the apical membrane.

Recent studies [8] have shown that apical constriction of mesoderm progenitors is achieved by pulsed contractions of an actomyosin cortical network at the apical side.

Interestingly, these pulsed contractions are asynchronous among neighboring cells. Between the successive contractions, which are controlled by the transcription factor Snail, the surface of each cell is stabilized so that it does not expand due to pulling by contracting neighbors. The stabilization, under the control of Twist, provides a 'ratchet' mechanism causing a net decrease of apical surface over time. The mechanical basis of cell surface stabilization between pulses is still unclear, but it is conceivable that, upon contraction, the cell changes its elastic modulus, e.g., through crosslinking of the apical actin network, or that the contracted state is stabilized by new cell–cell adhesions. Cell surface decrease is therefore achieved by sequential modifications of the cell's mechanical properties (Figure 3A).

Dorsal Closure

Dorsal closure is a well-studied morphogenetic process during which an opening in the dorsal epidermis of the *Drosophila* embryo is closed. The dorsal hole is filled with amnioserosa cells and surrounded by epidermal tissue. Closure results from the coordinated activity of epidermal cells moving in from the sides and amnioserosa cells constricting apically [93]. This process is controlled by a number of different signaling pathways and involves the establishment of a supracellular contractile actomyosin cable at the leading edge of the flanking epidermal cells and the formation of cellular protrusions in these cells.

Recent studies have shown that, similar to mesoderm progenitors during ventral furrow formation, the apical constriction of amnioserosa cells is not continuous, but pulsed [94]. As during ventral furrow formation, the pulsed contractions are not synchronous between neighbors and a ratchet mechanism has been proposed to account for the resulting global tissue contraction. The contraction of amnioserosa cells starts before the initiation of dorsal closure; however, prior to dorsal closure, the cells relax after each contraction pulse because of pulling forces exerted by neighboring cells. As a consequence, the leading edge of the epidermis undergoes small oscillations rather than closing the hole (Figure 3B). The beginning of dorsal closure coincides with a stiffening of this leading edge, apparently resulting from an increase in tension and/or stiffness of the supracellular actin cable surrounding the leading edge. The cable has been proposed to function in this process by acting as a ratchet, preventing complete relaxation of the pulsing cells and thus triggering global tissue contraction. Successive rows of amnioserosa cells are then sequentially stabilized in a contracted state, driving further contraction of the tissue. The surface stabilization mechanism is not known but is likely to involve an increase in cellular stiffness. Here again, global tissue remodeling is achieved by sequential modifications of the mechanical properties of the amnioserosa cells and of the surrounding epidermis (Figure 3B).

Convergent Extension

Convergent extension is a common process in vertebrate gastrulation and entails the narrowing of the forming embryonic axis along its mediolateral extent and concomitant elongation along its anterior-posterior axis [95]. In amphibians, convergent extension movements have been correlated with cells undergoing mediolateral cell intercalations. In principle, mediolateral cell intercalations could be the cause or the consequence of convergent extension movements, although the majority of experimental evidence supports the former assumption. Two different modes of mediolateral cell intercalations have been described so far — the bipolar mediolateral oriented mode, and the monopolar boundary-capturing mode. Cells undergoing bipolar mediolateral cell intercalations are thought to form protrusions oriented along the mediolateral axis and adhere with these protrusions to neighboring cells. This results in traction on their surfaces, which leads to cell elongation and eventually intercalation oriented along the mediolateral axis (Figure 3C).

Although mediolateral cell intercalations likely constitute the main force-generating process underlying convergent extension, direct evidence *in vivo* has been scarce. In recent studies, myosin II has been shown to be required for the formation of a cortical actin network in mesoderm cells undergoing mediolateral cell intercalations [96,97]. Interestingly, this cortical actin network consists of foci connected by actin cables, is polarized along the mediolateral axis, and undergoes pulsed contractions oriented parallel to this axis (Figure 3C). When myosin II activity is impaired, the cortical actin network is disrupted, cells fail to exhibit normal protrusive activity, and convergent extension movements are reduced. This suggests that oriented actomyosin-mediated contractions of the cortical actin network drive mediolateral cell intercalation during *Xenopus* gastrulation. However, interfering with myosin activity also affects cell properties other than contractility, such as cell adhesion, and therefore a mono-causal relationship between myosin-mediated cell contraction and mediolateral cell intercalation that triggers convergent extension movements cannot yet be concluded from these studies.

Outlook

We have only begun to understand the role of cell shape change in tissue morphogenesis during development. Biomechanical studies indicate that the shape of cells can be understood as the result of basic mechanical parameters such as cortical tension, cellular viscoelastic properties and cell adhesion. While several signaling pathways and effector molecules that control these mechanical properties have been identified, it also is becoming increasingly clear that numerous feedback loops exist from cellular mechanics back to protein expression and activity. It will be important to understand which mechanical properties are actively controlled by these feedback loops and how these control mechanisms function molecularly. Direct micromanipulation experiments, where the mechanical state of a cell is physically modified, will help identify proteins that are upregulated and/or activated in response to such treatments and which might therefore constitute integral parts of mechanosensing pathways.

Another open question is how individual cell shape change leads to global tissue morphogenesis. This question can be addressed through theoretical modeling in either a bottom-up approach, i.e. determining how individual cell shape change control tissue morphogenesis [94], or a top-down approach, i.e. analyzing how tissue morphogenesis correlates with different types of cell shape changes. Recent examples for top-down approaches include studies that have provided novel insight into the mechanisms of tissue deformation by automatically analyzing cell shape changes during embryogenesis [98–100].

The powerful approach of theoretical modeling paired with precise quantitative measurements will likely prove essential to understanding the contribution and regulation of specific

cellular parameters that produce cell shape change during tissue morphogenesis [94,101]. The main challenge in the future will be to develop tools to accurately measure and precisely modulate cellular mechanical properties *in vivo*, a precondition for producing meaningful and predictive theoretical models of tissue deformations in development.

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References

1. Thompson, D.A.W. (1917). On Growth and Form (Cambridge University Press).
2. Ingber, D.E. (1993). The riddle of morphogenesis: a question of solution chemistry or molecular cell engineering? Cell *75*, 1249–1252.
3. Bereiter-Hahn, J. (2005). Mechanics of crawling cells. Med. Eng. Phys. *27*, 743–753.
4. Eggert, U.S., Mitchison, T.J., and Field, C.M. (2006). Animal cytokinesis: from parts list to mechanisms. Annu. Rev. Biochem. *75*, 543–566.
5. Paluch, E., Sykes, C., Prost, J., and Bornens, M. (2006). Dynamic modes of the cortical actomyosin gel during cell locomotion and division. Trends Cell Biol. *16*, 5–10.
6. Lammermann, T., Bader, B.L., Monkley, S.J., Worbs, T., Wedlich-Soldner, R., Hirsch, K., Keller, M., Forster, R., Critchley, D.R., Fassler, R., *et al*. (2008). Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature *453*, 51–55.
7. Vicente-Manzanares, M., Zareno, J., Whitmore, L., Choi, C.K., and Horwitz, A.F. (2007). Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. J. Cell Biol. *176*, 573–580.
8. Martin, A.C., Kaschube, M., and Wieschaus, E.F. (2009). Pulsed contractions of an actin-myosin network drive apical constriction. Nature *457*, 495–499.
9. Borisy, G.G., and Svitkina, T.M. (2000). Actin machinery: pushing the envelope. Curr. Opin. Cell Biol. *12*, 104–112.
10. Rafelski, S.M., and Theriot, J.A. (2004). Crawling toward a unified model of cell mobility: spatial and temporal regulation of actin dynamics. Annu. Rev. Biochem. *73*, 209–239.
11. Mitchison, T.J., Charras, G.T., and Mahadevan, L. (2008). Implications of a poroelastic cytoplasm for the dynamics of animal cell shape. Semin. Cell Dev. Biol. *19*, 215–223.
12. Sheetz, M.P., Sable, J.E., and Döbereiner, H.G. (2006). Continuous membrane-cytoskeleton adhesion requires continuous accommodation to lipid and cytoskeleton dynamics. Annu. Rev. Biophys. Biomol. Struct. *35*, 417–434.
13. Keren, K., Pincus, Z., Allen, G.M., Barnhart, E.L., Marriott, G., Mogilner, A., and Theriot, J.A. (2008). Mechanism of shape determination in motile cells. Nature *453*, 475–480.
14. Effler, J.C., Kee, Y.S., Berk, J.M., Tran, M.N., Iglesias, P.A., and Robinson, D.N. (2006). Mitosis-specific mechanosensing and contractile-protein redistribution control cell shape. Curr. Biol. *16*, 1962–1967.
15. Ingber, D.E. (2003). Tensegrity I. Cell structure and hierarchical systems biology. J. Cell Sci. *116*, 1157–1173.
16. Thery, M., and Bornens, M. (2006). Cell shape and cell division. Curr. Opin. Cell Biol. *18*, 648–657.
17. Blaser, H., Reichman-Fried, M., Castanon, I., Dumstrei, K., Marlow, F.L., Kawakami, K., Solnica-Krezel, L., Heisenberg, C.P., and Raz, E. (2006). Migration of zebrafish primordial germ cells: a role for myosin contraction and cytoplasmic flow. Dev Cell *11*, 613–627.
18. Kunwar, P.S., Siekhaus, D.E., and Lehmann, R. (2006). In vivo migration: a germ cell perspective. Annu. Rev. Cell. Dev. Biol. *22*, 237–265.
19. Fraser, S.E., and Harland, R.M. (2000). The molecular metamorphosis of experimental embryology. Cell *100*, 41–55.
20. Scott, M.P. (2000). Development: the natural history of genes. Cell *100*, 27–40.
21. Montell, D.J. (2008). Morphogenetic cell movements: diversity from modular mechanical properties. Science *322*, 1502–1505.
22. Lecuit, T., and Lenne, P.F. (2007). Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. Nat. Rev. Mol. Cell. Biol. *8*, 633–644.
23. Farhadifar, R., Roper, J.C., Aigouy, B., Eaton, S., and Julicher, F. (2007). The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. Curr. Biol. *17*, 2095–2104.
24. Hutson, M.S., Tokutake, Y., Chang, M.S., Bloor, J.W., Venakides, S., Kiehart, D.P., and Edwards, G.S. (2003). Forces for morphogenesis investigated with laser microsurgery and quantitative modeling. Science *300*, 145–149.
25. Rauzi, M., Verant, P., Lecuit, T., and Lenne, P.F. (2008). Nature and anisotropy of cortical forces orienting Drosophila tissue morphogenesis. Nat. Cell. Biol. *10*, 1401–1410.
26. Toyama, Y., Peralta, X.G., Wells, A.R., Kiehart, D.P., and Edwards, G.S. (2008). Apoptotic force and tissue dynamics during Drosophila embryogenesis. Science *321*, 1683–1686.
27. Davidson, L., von Dassow, M., and Zhou, J. (2009). Multi-scale mechanics from molecules to morphogenesis. Int. J. Biochem. Cell Biol. DOI: 10.1016/j.biocel.2009.04.015.
28. Yeung, A., and Evans, E. (1989). Cortical shell-liquid core model for passive flow of liquid-like spherical cells into micropipets. Biophys J. *56*, 139–149.
29. Hochmuth, R.M. (2000). Micropipette aspiration of living cells. J. Biomech. *33*, 15–22.
30. Dai, J., Ting-Beall, H.P., Hochmuth, R.M., Sheetz, M.P., and Titus, M.A. (1999). Myosin I contributes to the generation of resting cortical tension. Biophys. J. *77*, 1168–1176.
31. Lomakina, E.B., Spillmann, C.M., King, M.R., and Waugh, R.E. (2004). Rheological analysis and measurement of neutrophil indentation. Biophys. J. *87*, 4246–4258.
32. Krieg, M., Arboleda-Estudillo, Y., Puech, P.H., Kafer, J., Graner, F., Muller, D.J., and Heisenberg, C.P. (2008). Tensile forces govern germ-layer organization in zebrafish. Nat. Cell. Biol. *10*, 429–436.
33. Liu, X.H., and Wang, X. (2004). The deformation of an adherent leukocyte under steady shear flow: a numerical study. J. Biomech. *37*, 1079–1085.
34. Caille, N., Thoumine, O., Tardy, Y., and Meister, J.J. (2002). Contribution of the nucleus to the mechanical properties of endothelial cells. J. Biomech. *35*, 177–187.
35. Riveline, D., Zamir, E., Balaban, N.Q., Schwarz, U.S., Ishizaki, T., Narumiya, S., Kam, Z., Geiger, B., and Bershadsky, A.D. (2001). Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. J. Cell Biol. *153*, 1175–1186.
36. Brodland, W.G., and Chen, H.H. (2000). The mechanics of cell sorting and envelopment. J. Biomech. *33*, 845–851.
37. Janmey, P.A., and McCulloch, C.A. (2007). Cell mechanics: integrating cell responses to mechanical stimuli. Annu. Rev. Biomed. Eng. *9*, 1–34.
38. Tabdanov, E., Borghi, N., Brochard-Wyart, F., Dufour, S., and Thiery, J.P. (2009). Role of E-cadherin in membrane-cortex interaction probed by nanotube extrusion. Biophys. J. *96*, 2457–2465.
39. Balaban, N.Q., Schwarz, U.S., Riveline, D., Goichberg, P., Tzur, G., Sabanay, I., Mahalu, D., Safran, S., Bershadsky, A., Addadi, L., *et al*. (2001). Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat. Cell Biol. *3*, 466–472.
40. Wang, N., and Ingber, D.E. (1994). Control of cytoskeletal mechanics by extracellular matrix, cell shape, and mechanical tension. Biophys. J. *66*, 2181–2189.
41. Oates, A.C., Gorfinkiel, N., Gonzalez-Gaitan, M., and Heisenberg, C.P. (2009). Quantitative approaches in developmental biology. Nat. Rev. Genet. *10*, 517–530.
42. Steinberg, M.S. (1963). Reconstruction of tissues by dissociated cells. Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation. Science *141*, 401–408.
43. Foty, R.A., Pfleger, C.M., Forgacs, G., and Steinberg, M.S. (1996). Surface tensions of embryonic tissues predict their mutual envelopment behavior. Development *122*, 1611–1620.
44. Foty, R.A., and Steinberg, M.S. (2005). The differential adhesion hypothesis: a direct evaluation. Dev. Biol. *278*, 255–263.
45. Bertet, C., Sulak, L., and Lecuit, T. (2004). Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. Nature *429*, 667–671.
46. Blankenship, J.T., Backovic, S.T., Sanny, J.S., Weitz, O., and Zallen, J.A. (2006). Multicellular rosette formation links planar cell polarity to tissue morphogenesis. Dev. Cell *11*, 459–470.
47. Wei, Y., and Mikawa, T. (2000). Formation of the avian primitive streak from spatially restricted blastoderm: evidence for polarized cell division in the elongating streak. Development *127*, 87–96.
48. da Silva, S.M., and Vincent, J.P. (2007). Oriented cell divisions in the extending germband of Drosophila. Development *134*, 3049–3054.
49. Gong, Y., Mo, C., and Fraser, S.E. (2004). Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. Nature *430*, 689–693.
50. Ridley, A.J., Schwartz, M.A., Burridge, K., Firtel, R.A., Ginsberg, M.H., Borisy, G., Parsons, J.T., and Horwitz, A.R. (2003). Cell migration: integrating signals from front to back. Science *302*, 1704–1709.
51. Paluch, E., and Charras, G.T. (2008). Blebs lead the way: how to migrate without lamellipodia. Nat. Rev. Mol. Cell Biol. *9*, 730–736.
52. Lauffenburger, D.A., and Horwitz, A.F. (1996). Cell migration: a physically integrated molecular process. Cell *84*, 359–369.
53. Mitchison, T.J., and Cramer, L.P. (1996). Actin-based cell motility and cell locomotion. Cell *84*, 371–379.

54. Giannone, G., Dubin-Thaler, B.J., Rossier, O., Cai, Y., Chaga, O., Jiang, G., Beaver, W., Dobereiner, H.G., Freund, Y., Borisy, G., *et al*. (2007). Lamellipodial actin mechanically links myosin activity with adhesion-site formation. Cell *128*, 561–575.

55. Palecek, S.P., Loftus, J.C., Ginsberg, M.H., Lauffenburger, D.A., and Horwitz, A.F. (1997). Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature *385*, 537–540.

56. Bellion, A., Baudoin, J.-P., Alvarez, C., Bornens, M., and Metin, C. (2005). Nucleokinesis in tangentially migrating neurons comprises two alternating phases: forward migration of the Golgi/centrosome associated with centrosome splitting and myosin contraction at the rear. J. Neurosci. *25*, 5691–5699.

57. Pollard, T.D., and Borisy, G.G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. Cell *112*, 453–465.

58. Kunwar, P.S., Sano, H., Renault, A.D., Barbosa, V., Fuse, N., and Lehmann, R. (2008). Tre1 GPCR initiates germ cell transepithelial migration by regulating Drosophila melanogaster E-cadherin. J. Cell. Biol. *183*, 157–168.

59. Friedl, P., and Gilmour, D. (2009). Collective cell migration in morphogenesis, regeneration and cancer. Nat. Rev. Mol. Cell Biol. *10*, 445–457.

60. Melani, M., Simpson, K.J., Brugge, J.S., and Montell, D. (2008). Regulation of cell adhesion and collective cell migration by hindsight and its human homolog RREB1. Curr. Biol. *18*, 532–537.

61. McDonald, J.A., Khodyakova, A., Aranjuez, G., Dudley, C., and Montell, D.J. (2008). PAR-1 kinase regulates epithelial detachment and directional protrusion of migrating border cells. Curr. Biol. *18*, 1659–1667.

62. Niewiadomska, P., Godt, D., and Tepass, U. (1999). DE-Cadherin is required for intercellular motility during Drosophila oogenesis. J. Cell Biol. *144*, 533–547.

63. Chen, X., and Gumbiner, B.M. (2006). Paraxial protocadherin mediates cell sorting and tissue morphogenesis by regulating C-cadherin adhesion activity. J. Cell Biol. *174*, 301–313.

64. Zhong, Y., Brieher, W.M., and Gumbiner, B.M. (1999). Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. J. Cell Biol. *144*, 351–359.

65. Wacker, S., Grimm, K., Joos, T., and Winklbauer, R. (2000). Development and control of tissue separation at gastrulation in Xenopus. Dev. Biol. *224*, 428–439.

66. Medina, A., Swain, R.K., Kuerner, K.M., and Steinbeisser, H. (2004). Xenopus paraxial protocadherin has signaling functions and is involved in tissue separation. EMBO J. *23*, 3249–3258.

67. Unterseher, F., Hefele, J.A., Giehl, K., De Robertis, E.M., Wedlich, D., and Schambony, A. (2004). Paraxial protocadherin coordinates cell polarity during convergent extension via Rho A and JNK. EMBO J. *23*, 3259–3269.

68. Nandadasa, S., Tao, Q., Menon, N.R., Heasman, J., and Wylie, C. (2009). N- and E-cadherins in Xenopus are specifically required in the neural and non-neural ectoderm, respectively, for F-actin assembly and morphogenetic movements. Development *136*, 1327–1338.

69. Marsden, M., and DeSimone, D.W. (2003). Integrin-ECM interactions regulate cadherin-dependent cell adhesion and are required for convergent extension in Xenopus. Curr. Biol. *13*, 1182–1191.

70. Dzamba, B.J., Jakab, K.R., Marsden, M., Schwartz, M.A., and DeSimone, D.W. (2009). Cadherin adhesion, tissue tension, and noncanonical Wnt signaling regulate fibronectin matrix organization. Dev. Cell *16*, 421–432.

71. Davidson, L.A., Marsden, M., Keller, R., and Desimone, D.W. (2006). Integrin alpha5beta1 and fibronectin regulate polarized cell protrusions required for Xenopus convergence and extension. Curr. Biol. *16*, 833–844.

72. Somogyi, K., and Rorth, P. (2004). Evidence for tension-based regulation of Drosophila MAL and SRF during invasive cell migration. Dev. Cell *7*, 85–93.

73. Lecaudey, V., Cakan-Akdogan, G., Norton, W.H., and Gilmour, D. (2008). Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. Development *135*, 2695–2705.

74. Aman, A., and Piotrowski, T. (2008). Wnt/beta-catenin and Fgf signaling control collective cell migration by restricting chemokine receptor expression. Dev. Cell *15*, 749–761.

75. Nechiporuk, A., and Raible, D.W. (2008). FGF-dependent mechanosensory organ patterning in zebrafish. Science *320*, 1774–1777.

76. Bianco, A., Poukkula, M., Cliffe, A., Mathieu, J., Luque, C.M., Fulga, T.A., and Rorth, P. (2007). Two distinct modes of guidance signalling during collective migration of border cells. Nature *448*, 362–365.

77. McDonald, J.A., Pinheiro, E.M., and Montell, D.J. (2003). PVF1, a PDGF/VEGF homolog, is sufficient to guide border cells and interacts genetically with Taiman. Development *130*, 3469–3478.

78. Montero, J.A., Kilian, B., Chan, J., Bayliss, P.E., and Heisenberg, C.P. (2003). Phosphoinositide 3-kinase is required for process outgrowth and cell polarization of gastrulating mesendodermal cells. Curr. Biol. *13*, 1279–1289.

79. Nagel, M., Tahinci, E., Symes, K., and Winklbauer, R. (2004). Guidance of mesoderm cell migration in the Xenopus gastrula requires PDGF signaling. Development *131*, 2727–2736.

80. Yang, X., Chrisman, H., and Weijer, C.J. (2008). PDGF signalling controls the migration of mesoderm cells during chick gastrulation by regulating N-cadherin expression. Development *135*, 3521–3530.

81. Simons, M., and Mlodzik, M. (2008). Planar cell polarity signaling: from fly development to human disease. Annu. Rev. Genet. *42*, 517–540.

82. Tada, M., and Kai, M. (2009). Noncanonical Wnt/PCP signaling during vertebrate gastrulation. Zebrafish *6*, 29–40.

83. Bastock, R., and Strutt, D. (2007). The planar polarity pathway promotes coordinated cell migration during Drosophila oogenesis. Development *134*, 3055–3064.

84. Marlow, F., Topczewski, J., Sepich, D., and Solnica-Krezel, L. (2002). Zebrafish rho kinase 2 acts downstream of wnt11 to mediate cell polarity and effective convergence and extension movements. Curr. Biol. *12*, 876–884.

85. Ulrich, F., Krieg, M., Schotz, E.M., Link, V., Castanon, I., Schnabel, V., Taubenberger, A., Mueller, D., Puech, P.H., and Heisenberg, C.P. (2005). Wnt11 functions in gastrulation by controlling cell cohesion through Rab5c and E-cadherin. Dev. Cell *9*, 555–564.

86. Wodarz, A., Stewart, D.B., Nelson, W.J., and Nusse, R. (2006). Wingless signaling modulates cadherin-mediated cell adhesion in Drosophila imaginal disc cells. J. Cell. Sci. *119*, 2425–2434.

87. Winter, C.G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J.D., and Luo, L. (2001). Drosophila Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. Cell *105*, 81–91.

88. Silver, D.L., and Montell, D.J. (2001). Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in Drosophila. Cell *107*, 831–841.

89. Beccari, S., Teixeira, L., and Rorth, P. (2002). The JAK/STAT pathway is required for border cell migration during Drosophila oogenesis. Mech. Dev. *111*, 115–123.

90. Yamashita, S., Miyagi, C., Carmany-Rampey, A., Shimizu, T., Fujii, R., Schier, A.F., and Hirano, T. (2002). Stat3 controls cell movements during zebrafish gastrulation. Dev. Cell *2*, 363–375.

91. Miyagi, C., Yamashita, S., Ohba, Y., Yoshizaki, H., Matsuda, M., and Hirano, T. (2004). STAT3 noncell-autonomously controls planar cell polarity during zebrafish convergence and extension. J. Cell Biol. *166*, 975–981.

92. Leptin, M. (1999). Gastrulation in Drosophila: the logic and the cellular mechanisms. EMBO J. *18*, 3187–3192.

93. Jacinto, A., Woolner, S., and Martin, P. (2002). Dynamic analysis of dorsal closure in Drosophila: from genetics to cell biology. Dev. Cell *3*, 9–19.

94. Solon, J., Kaya, A., Colombelli, J., and Brunner, D. (2009). Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. Cell *137*, 1183–1185.

95. Keller, R. (2002). Shaping the vertebrate body plan by polarized embryonic cell movements. Science *298*, 1950–1954.

96. Rolo, A., Skoglund, P., and Keller, R. (2009). Morphogenetic movements driving neural tube closure in Xenopus require myosin IIB. Dev. Biol. *327*, 327–338.

97. Skoglund, P., Rolo, A., Chen, X., Gumbiner, B.M., and Keller, R. (2008). Convergence and extension at gastrulation require a myosin IIB-dependent cortical actin network. Development *135*, 2435–2444.

98. Blanchard, G.B., Kabla, A.J., Schultz, N.L., Butler, L.C., Sanson, B., Gorfinkiel, N., Mahadevan, L., and Adams, R.J. (2009). Tissue tectonics: morphogenetic strain rates, cell shape change and intercalation. Nat. Methods *6*, 458–464.

99. Gorfinkiel, N., Blanchard, G.B., Adams, R.J., and Martinez-Arias, A. (2009). Mechanical control of global cell behaviour during dorsal closure in Drosophila. Development *136*, 1889–1898.

100. Butler, L.C., Blanchard, G.B., Kabla, A.J., Lawrence, N.J., Welchman, D.P., Mahadevan, L., Adams, R.J., and Sanson, B. (2009). Cell shape changes indicate a role for extrinsic tensile forces in Drosophila germ-band extension. Nat. Cell Biol. *11*, 859–864.

101. Riedel-Kruse, I.H., Muller, C., and Oates, A.C. (2007). Synchrony dynamics during initiation, failure, and rescue of the segmentation clock. Science *317*, 1911–1915.

102. Reichman-Fried, M., Minina, S., and Raz, E. (2004). Autonomous modes of behavior in primordial germ cell migration. Dev. Cell *6*, 589–596.

103. Thorpe, J.L., Doitsidou, M., Ho, S.Y., Raz, E., and Farber, S.A. (2004). Germ cell migration in zebrafish is dependent on HMGCoA reductase activity and prenylation. Dev. Cell *6*, 295–302.

104. Haas, P., and Gilmour, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. Dev. Cell *10*, 673–680.

105. Paluch, E., and Heisenberg, C.-P. (2009). Chaos begets order: asynchronous cell contractions drive epithelial morphogenesis. Dev. Cell *16*, 4–6.

106. Helenius, J., Heisenberg, C.P., Gaub, H.E., and Muller, D.J. (2008). Single-cell force spectroscopy. J. Cell Sci. *121*, 1785–1791.

107. Evans, E., Ritchie, K., and Merkel, R. (1995). Sensitive force technique to probe molecular adhesion and structural linkages at biological interfaces. Biophys. J. *68*, 2580–2587.

108. Sung, K.-L.P., Sung, L.A., Crimmins, M., Burakoff, S.J., and Chien, S. (1986). Determination of junction avidity of cytolytic T cell and target cell. Science *234*, 1405–1408.

109. Chu, Y.S., Thomas, W.A., Eder, O., Pincet, F., Perez, E., Thiery, J.P., and Dufour, S. (2004). Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. J. Cell. Biol. *167*, 1183–1194.

110. Benoit, M., Gabriel, D., Gerisch, G., and Gaub, H.E. (2000). Discrete interactions in cell adhesion measured by single-molecule force spectroscopy. Nat. Cell Biol. *2*, 313–317.

111. Kucik, D.F. (2009). Measurement of adhesion under flow conditions. Curr. Protoc. Cell Biol. Chapter 9, Unit 9.6.

112. Puech, P.H., Taubenberger, A., Ulrich, F., Krieg, M., Muller, D.J., and Heisenberg, C.P. (2005). Measuring cell adhesion forces of primary gastrulating cells from zebrafish using atomic force microscopy. J. Cell Sci. *118*, 4199–4206.

113. Litvinov, R.I., Shuman, H., Bennett, J.S., and Weisel, J.W. (2002). Binding strength and activation state of single fibrinogen-integrin pairs on living cells. Proc. Natl. Acad. Sci. USA *99*, 7426–7431.

114. Evans, E., and Yeung, A. (1989). Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. Biophys. J. *56*, 151–160.

115. Wu, H.W., T,K., and Moy, V.T. (1998). Mechanical properties of L929 cells measured by atomic force microscopy: effects of anticytoskeletal drugs and membrane crosslinking. Scanning *20*, 389–397.

116. Thoumine, O., and Ott, A. (1997). Time scale dependent viscoelastic and contractile regimes in fobroblasts probed by microplate manipulation. J. Cell Sci. *110*, 2109–2116.

117. Guck, J., Ananthakrishnan, R., Mahmood, H., Moon, T.J., Cunningham, C.C., and Kas, J. (2001). The optical stretcher: a novel laser tool to micromanipulate cells. Biophys. J. *81*, 767–784.