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Tuning cell shape change with contractile ratchets

Frank M. Mason and Adam C. Martin

Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Abstract

Throughout the lifespan of an organism, shape changes are necessary for cells to carry out their essential functions. Nowhere is this more dramatic than embryonic development and gastrulation, when cell shape changes drive large-scale rearrangements in tissue architecture to establish the body plan of the organism. A longstanding question for both cell and developmental biologists has been how are forces generated to change cell shape? Recent studies in both cell culture and developing embryos have combined live imaging, computational analysis, genetics, and biophysics to identify ratchet-like behaviors in actomyosin networks that operate to incrementally change cell shape, drive cell movement, and deform tissues. Our analysis of several cell shape changes lead us to propose four regulatory modules associated with ratchet-like mediated deformations that are tuned to generate diverse cell behaviors, coordinating cell shape change across a tissue.

Keywords

force; morphogenesis; cell shape change; cell migration; actin; myosin; adherens junctions; E-cadherin; apical constriction; gastrulation; intercalation; convergence; extension

Introduction

How do cells generate force to change their shape and the shape of a tissue? This question has intrigued biologists for decades, ever since the observation that cell shape changes contribute to tissue deformation during gastrulation [1]. We now know that networks of filamentous actin (F-actin) and the molecular motor, type II myosin (Myo-II) play a predominant role in generating forces that change cell shape and deform tissues during development [2]. Actomyosin networks generate contractile force through the activity of Myo-II, which assembles into bipolar minifilaments containing 15–30 motor heads that pull actin filaments relative to each other to reorganize F-actin networks and generate tension [3]. When coupled to cell-substrate or cell-cell adhesions, contractile actomyosin networks transmit forces to their environment. Contractile forces result in a wide array of cell shape changes that occur during development [2]. Therefore it is surprising that recent live imaging of three different cell behaviors, cell migration, apical constriction, and cell intercalation, revealed a common ratchet-like mechanism that drives cell shape change. Here, we review how forces drive these cell shape changes and propose that diverse cell behaviors can result

by tuning four key regulatory modules of the ratchet-like mechanism: localization, pulsation, adhesion, and stabilization.

Forces that drive cell shape change in vitro: Cell migration

Cell migration requires membrane protrusion to advance the leading edge of the cell and contraction to pull the remaining cell body forward (Figure 1a) [4]. Protrusion is driven by the polymerization of a dense network of F-actin filaments called the lamellipodium in which actin filaments are mostly oriented with their growing (plus or barbed) ends facing the plasma membrane (Figure 1b) [5–8]. Contractile forces are generated by a Myo-II-containing network of longer, more randomly oriented actin filaments behind the lamellipodium, called the lamella, and by actomyosin bundles called stress fibers (Figure 1b) [5,9–11]. F-actin networks must be coupled to the underlying substrate for protrusive and contractile forces to change cell shape rather than cause F-actin retrograde flow (Figure 1a) [12]. Cell-substrate adhesion and traction is mediated by integrin-containing adhesion complexes, which appear in the lamellipodium as nascent adhesions and a subset of which mature into focal adhesions at the border between the lamellipodium and the lamella [7,13]. The coupling between actin networks and adhesions appears to be locally regulated such that it acts as a molecular ‘clutch’, where maximum protrusion force (from F-actin polymerization) and traction force (from actomyosin contraction) is promoted by tight coupling (clutch engagement) between the cytoskeleton and the substrate [14,15]. Optimal cell migration requires a proper balance between contractility and cell-substrate adhesion because cells require traction to move, but too much adhesion results in drag forces that resist actomyosin contraction [16].

Live imaging and quantitative analysis of several cell types has highlighted an important role for cycles of contraction during cell migration. Spreading mouse embryonic fibroblasts (MEFs) exhibit Myo-II-dependent periodic contractions every ~ 25 seconds that transiently interrupt protrusion and retract the leading edge of the cell [17,18]. In addition, other cell types also exhibit periodic contractions that pause leading edge advance, with periods ranging from 1 to 10 minutes [19–21]. Periodic contractions are associated with the condensation of F-actin and Myo-II into actin bundles, or actin arcs, that are oriented parallel to the membrane [5,10,18,19,22]. A recent study using photoconvertible fluorescent actin to follow F-actin turnover directly observed lamellipodial F-actin networks being transformed into actin arcs during membrane retraction [19]. This suggests that Myo-II periodically collapses the lamellipodial F-actin network, generating contractile forces that cause the leading edge of the migrating cell to periodically retract.

If actomyosin contraction continually counters leading edge advance, how does this behavior promote effective migration? Focal adhesion maturation is stimulated by mechanical tension and periodic Myo-II contractions stabilize nascent focal adhesions at the border between the lamellipodium and the lamella [13,18,21,23]. As a result, focal adhesions can exhibit a periodic distribution relative to the advancing cell edge [17]. Maturing focal adhesions appear to stop the retrograde flow of actin arcs, functioning as the ‘clutch’ to anchor arcs relative to the substrate [19]. The coupling of actin arcs with focal adhesions prevents further retraction and serves as a mechanical base for the subsequent

protrusion. This supports a hypothesis that leading edge advance acts in a cyclical and ratchet-like manner: membrane extension initiates new focal adhesions that engage actin arcs and establish a new base for membrane protrusion (Figure 1c) [19]. Importantly, the engagement of the ‘clutch’ between F-actin networks, focal adhesions, and the substrate can be modulated, with the strength of this coupling being correlated with the magnitude of the traction force [12]. Thus, the magnitude of a cell shape change can be tuned by regulating the extent to which this ‘clutch’ is engaged.

Forces that drive cell shape change during gastrulation: Apical constriction

In epithelial sheets, the mechanical coupling of cells via adherens junctions allows individual cell behaviors to propagate change across the tissue, leading to tissue morphogenesis. A common epithelial cell shape change that is involved in morphogenetic events throughout development is apical constriction [24]. Apical constriction reduces the apical surface area of the epithelial cell, which contains adherens junctions, and often expands at the basal end, transforming a columnar-shaped cell to a wedge or cone shape (Figure 2a). The presence of a population of interconnected wedge-shaped cells in an epithelial sheet is thought to promote epithelial bending and cell invagination [25]. An excellent model system to study this process is *Drosophila* gastrulation, where apical constriction of prospective mesoderm cells appears to drive the invagination of these cells into the interior of the embryo (Figure 2b) [26,27].

Apical constriction requires contractile forces to pull adherens junctions at the cell circumference inwards. Early models of apical constriction suggested that shortening of a circumferential bundle of F-actin and Myo-II underlying the adherens junctions constricts epithelial cells like drawing a purse-string [28]. However, actin and Myo-II have also been observed across the entire apical cortex of *Drosophila* mesoderm cells [29,30]. Recently, live imaging of both Myo-II and cell boundaries revealed that Myo-II is present in a dynamic supracellular meshwork across the apical surface of mesoderm cells (Figure 2c) [31]. Myo-II appears as spots that coalesce to form larger Myo-II aggregates and bundle-like fibers on the medial apical surface of the cell. Myo-II coalescence is correlated with apical constriction and the inward bending of adherens junctions, suggesting that the Myo-II spot movement represents contraction of the F-actin network spanning the apical surface (Figure 2d) [31]. Disruption of adherens junctions causes cellular actomyosin networks to contract away from the cell circumference and cells fail to undergo apical constriction, demonstrating that, similar to cell migration, engagement of a ‘clutch’ between actomyosin networks and the adhesion machinery is critical for cell shape change [29,32,33].

Apical constriction and Myo-II contraction in mesoderm cells are not continuous, but occur as a series of constriction pulses that are often asynchronous in neighboring cells [31]. Individual cells undergo three to five pulses before invagination that exhibit a periodicity of ~90 seconds. Importantly, mesoderm cells maintain their contracted state between contraction pulses, which allows these cells to rapidly (5 to 10 minutes) reduce their apical area by 75% in an incremental manner. This stabilization of the contracted state between pulses occurs despite the fact that apical constriction generates levels of tension along the length of the furrow that are high enough to influence the directionality of the cell shape

change (Figure 2b and 2c) [32]. Thus, it appears that apical constriction also occurs in a ratchet-like manner: pulses of actomyosin contraction drive apical area reduction and cell shape is stabilized between pulses to prevent apical relaxation (Figure 2d).

What stabilizes cell shape during pulsed contraction? Insight into this question comes from the observation that the transcription factor Twist is required for the stabilization component of pulsed contraction. Cells with insufficient Twist undergo pulsed contractions, but these contractions are not stabilized resulting in cell shape fluctuations without net constriction [31]. In wild-type embryos, Myo-II persists in cells after contraction pulses, leading to a gradual increase in Myo-II levels and the formation of a supracellular meshwork of actomyosin fibers. In the absence of Twist, Myo-II fails to persist at the apical cortex and the formation of the supracellular meshwork is disrupted [32]. Thus, actomyosin fibers running across the apical surface of mesoderm cells could act as a molecular ‘catch’ that prevent expansion and stabilize the area of the apical surface after a contraction pulse. Importantly, Twist-mediated Myo-II stabilization is required to generate tension across the tissue [32]. Tension possibly enhances apical Myo-II recruitment, which would lead to the rapid assembly of a supracellular actomyosin meshwork [34]. Unexpectedly, tension in the tissue is anisotropic, being highest along the embryo’s anterior-posterior axis where there is the least tissue movement (Figure 2b and 2c). This tension anisotropy causes cells to predominantly constrict along the orthogonal axis, resulting in wedge shaped cells that form a long, narrow furrow [32]. Thus, the stabilization or ‘catch’ component of apical constriction translates cell contraction into tissue-wide tension that influences the morphology of both cells and the tissue.

The efficient stabilization of apical contractions in the mesoderm contrasts with the inefficient dampening of cell shape fluctuations that occur in late *Drosophila* embryogenesis during dorsal closure. Apical constriction of amnioserosa cells during dorsal closure involves pulses of apical constriction that are slowly (2–3 hours) stabilized over many cycles of contraction [35–37]. Thus, regulating the engagement of a molecular ‘catch’ could be a mechanism to adjust the developmental timing of morphogenetic processes.

Forces that drive cell shape change during gastrulation: Cell intercalation

Convergence and extension movements elongate the embryo along one axis and contract it along the orthogonal axis. The resulting tissue flows are essential for gastrulation in many organisms and are driven by several different processes, including cell migration, cell-cell intercalation, and cell shape change [38,39]. In epithelial sheets, cell-cell intercalation results from cell reorganization in the plane of the epithelia, as seen during germband extension in *Drosophila* (Figure 3a and 3b) [40]. *Drosophila* germband cells are planar polarized, such that cell-cell interfaces between anterior-posterior neighbors (a-p interfaces) shrink, bringing cells together along the dorsal-ventral (d-v) axis and separating cells along the a-p axis. This can occur for isolated a-p interfaces, resulting in a 4-way vertex, and also for several aligned interfaces, resulting in a rosette structure (Figure 3a) [41,42]. These a-p interfaces contain higher levels of F-actin and Myo-II, which form multicellular cables, while d-v interfaces contain higher levels of adherens junction proteins (Figure 3c) [41–43].

This planar polarity results in anisotropic tension around the cell circumference, with tension being highest in shrinking a-p edges [44,45].

Although anisotropic tension itself could explain cell rearrangements observed during germband extension [45], recent live imaging of Myo-II revealed that pulsatile contractions are also present in the medial network spanning the apical surface [46–48]. Bursts of medial Myo-II coalescence correlate with fluctuations in apical area, similar to mesoderm cells [46,48]. Furthermore, Myo-II coalescence correlates with fluctuations in the length of a-p cell interfaces, with interfaces exhibiting a step-wise decrease in length [47]. Myo-II recruitment to a-p junctions follows medial contraction pulses and the shrinkage of the interface, suggesting that it does not initiate this shortening. Instead, interfaces without this Myo-II cable relax back to their previous length, suggesting that tension in the Myo-II cable stabilizes fluctuations that are elicited by the medial actomyosin meshwork [47]. Thus, the Myo-II cable along a-p interfaces serves as the ‘catch’ in a ratchet-like mechanism that incrementally shrinks interfaces of a given orientation, resulting in cell rearrangements (Figure 3d). Importantly, tension promotes the formation of multicellular Myo-II cables during rosette formation, suggesting that mechanical feedback regulates this activity to coordinate cellular behavior and generate tissue-level forces [44]. Mutants in the JAK/STAT signaling pathway fail to assemble junctional actomyosin cables and instead accumulate Myo-II in the medial meshwork. This results in apical constriction rather than cell intercalation, demonstrating that the localization of the contractile apparatus can be modulated to promote different cell shape changes [49].

Shape change in germband cells can also be influenced by the localization of the molecular ‘clutch’ that couples F-actin meshworks between cells. In addition to intercalation, germband cells also elongate along the a-p axis, which could be a passive response to concurrent morphogenetic movements [50]. Medial Myo-II coalescence exhibits planar polarized flows along the a-p axis, which depends on planar polarization of adherens junction proteins [46,47]. This flow could represent actomyosin meshwork contraction that preferentially exerts tension along the d-v axis to bias their elongation in the a-p direction [46,48]. Myo-II also exhibits flow into a-p junctions, suggesting that junctional actomyosin cables might actually be derived from the medial mesh, similar to the formation of actin arcs from lamellipodial actin meshworks [47]. Interestingly, F-actin polymerization and Myo-II are required for E-cadherin clustering and endocytosis in a-p junctions, suggesting that cross-talk exists between the actomyosin ratchet and junctional or ‘clutch’ remodeling, which would reinforce cell planar polarization [51].

Tuning cell shape change and tissue morphogenesis

Clear parallels exist when comparing the force generating mechanisms that drive cell migration, apical constriction, and cell intercalation. Each of these cell shape changes involves cyclical contraction and/or polymerization of dynamic actin meshworks that presumably result in cycles of force generation. These cytoskeletal forces are translated into cell shape change by the regulated coupling of the cytoskeleton to the plasma membrane, substrate, and adjacent cells through a molecular ‘clutch’ that consists of adhesion complexes. The result is pulsatile, sometime oscillatory, fluctuations in cell shape. Cell

shape change is sustained by stabilizing fluctuations, often through the use of actomyosin cables as a molecular ‘catch’ to prevent relaxation, such that they result in incremental change, a mechanism similar to that of a ratchet. Stabilization of cell shape fluctuations is critical to propagate forces across a tissue, leading to tissue morphogenesis.

Why do cells use a ratchet-like mechanism to change shape? We propose that this mechanism provides cells with four key regulatory modules that can be tuned by biochemical and mechanical signals to generate diverse cell shape changes and tissue morphologies. (1) Localization. Subcellular localization of either actomyosin bundles/meshworks and/or its attachment to the adhesion machinery dictates the type of cell shape change. Such is the difference between mesoderm invagination and germband extension, where differential positioning of actomyosin bundles leads to apical constriction or cell intercalation (Figure 4a). (2) Pulsation. The frequency and duration of pulses modulates the number of steps required for cell shape change. During cell migration, different cell types exhibit different retraction frequencies, thus different levels of persistent migration (Figure 4b). (3) Adhesion. Regulation of the molecular ‘clutch’ that couples the actomyosin network to adhesive structures modulates traction forces that are transmitted to the external environment. Traction is required to translate cytoskeletal forces into cell shape change and to transmit forces across a tissue (Figure 4c). (4) Stabilization. The extent of relaxation or engagement of a molecular ‘catch’ between fluctuations can determine the net speed of cell shape change. Regulation of this step causes mesoderm cells to constrict much faster than amnioserosa cells, even though the dynamics of the actomyosin meshworks are quite similar (Figure 4d). In summary, tuning localization, pulsation, adhesion, and stabilization would provide a means by which to generate a wide diversity of cell behaviors using a common force-generating machine. The responsiveness of these modules to forces would allow cells to collectively adapt to the changing mechanical landscape of a developing embryo, making morphogenetic movements robust to perturbation or variation [52].

Concluding perspectives

The conservation of the ratchet-like behavior observed during cell migration, constriction and intercalation establishes the importance of this molecular machine. However, many questions still remain about this mechanism. While it is clear that dynamic and stable actomyosin networks are important for inducing and sustaining cell shape change, the connections between these distinct types of networks is still unclear. Continued use and development of quantitative fluorescent speckle imaging, photoconvertible fluorescent proteins, and super-resolution microscopy to connect cytoskeletal organization and flows to quantitative measurements of traction force, and eventually intracellular forces, will be important to define the temporal progression of molecular components during cell shape change [53]. In addition, techniques that allow precise temporal and spatial control of protein activity will be important to separate the contribution of different subcellular pools of factors like Myo-II or adhesion proteins to shape change [54]. Finally, understanding how cell shape changes collectively drive tissue deformation will require determining how the regulatory modules outlined here are tuned by developmental and mechanical signals and in turn how the resulting forces influence transcriptional activity and cell fate [23,34,44,55–58]. Given the continual development of new imaging and analytical techniques and their

combination with quantitative models of collective cell behavior, we expect that the next few years will bring many more exciting findings regarding cell and tissue morphogenesis.

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Figure 1.

Forces that drive cell migration. (a) Subcellular localization of forces during cell migration. Forward directed red arrows indicate protrusive forces that result from actin polymerization and rearward arrows represent the corresponding retrograde flow of the network. Green arrows represent contractile forces in the lamella. (b) Magnified view of F-actin networks in the lamellipodium and the lamella. (c) Ratchet model for cell migration. F-actin polymerization generates protrusive force to push the plasma membrane forward (red arrows). Actomyosin contraction collapses the lamellipodial network into an actin arc (green

arrows), causing leading edge retraction. Rearward moving actin arcs are engaged by maturing focal adhesions, which serve as a molecular ‘clutch’ between actin networks and the substrate. The anchored actin arc becomes the mechanical base for a subsequent protrusion (green asterisks), resulting in a step-wise advance of the leading edge.

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Figure 2.

Forces that drive apical constriction during gastrulation. (a) Illustration of apical constriction. A polarized, columnar epithelial cell constricts its apical end and expands its basal end to become wedge-shaped. Apical constriction is driven by contractile force on the apical surface (green arrows). (b) *Drosophila* mesoderm invagination. Apical constriction of mesoderm cells on the ventral side of the embryo (top) drives epithelial folding and cell invagination. During invagination, tension is highest along the anterior-posterior axis of the embryo (red arrows). (c) Surface view of apical Myo-II (green) and cell outlines (magenta). Myo-II forms a supracellular meshwork. Apical constriction occurs predominantly along the ventral-lateral axis (horizontal), which pulls the surrounding tissue towards the ventral

midline (black arrows). (d) Ratchet model for apical constriction. Myo-II is recruited to an F-actin meshwork that spans the apical surface. Pulses of actomyosin network contraction pull adherens junctions inward, reducing apical area. Between pulses, cell shape does not relax, but is maintained, possibly by actomyosin fibers bridging the apical surface. This cycle is repeated to incrementally constrict the cell.

Figure 3.

Forces that drive cell intercalation during gastrulation. (a) Cell rearrangements that accompany *Drosophila* germband extension. Individual anterior-posterior (a-p) interfaces (top) can shrink to generate 4-way vertices, or Type 2 junctions, that are directionally resolved to bring cells together on the dorsal-ventral axis. In addition, multiple a-p interfaces (bottom) can shrink together to form multicellular rosettes that are also directionally resolved to extend the tissue. (b) Germband extension in *Drosophila*. (c) Planar polarization of Myo-II and a junctional protein, Baz/Par-3 in germband cells. Image courtesy of R. Fernandez-Gonzalez and J. Zallen. (d) Ratchet model for cell interaction. Actomyosin contractions on the medial apical surface reduce apical area and shrink vertical interfaces (red edges). Flow of Myo-II into the vertical junctions increases Myo-II intensity in the junctional cable. This stabilizes the interface by preventing it from lengthening. The process repeats to incrementally bring cells closer along the dorsal-ventral axis.

Figure 4.

Four regulatory modules associated with ratchet-like cell shape change. (a) Localization. The subcellular localization of contractile actomyosin networks dictates the type of cell shape change that occurs. In mesoderm cells, Myo-II is predominantly on the medial apical surface, resulting in apical constriction, whereas in germband cells, Myo-II is most abundant in planar polarized junctional cables, resulting in cell intercalation. (b) Pulsation. The frequency of contractions can regulate the persistence of a cell shape change. During cell migration, infrequent retractions allow more persistent migration whereas frequent retractions might allow a cell to probe its environment. (c) Adhesion. To translate internal

forces into cell shape change, the actin cytoskeleton must be coupled to neighboring cells or the substrate and generate traction. This coupling can be regulated by a molecular 'clutch'. When the clutch is released actin networks undergo retrograde flow. When the clutch is engaged, protrusive and contractile forces result in cell shape change and tissue-level forces. (d) Stabilization. Fluctuations in cell shape that result from pulsation must be sustained to drive rapid cell and tissue deformation. This is regulated by a molecular 'catch' that prevents cell relaxation after cell shape fluctuates. When this 'catch' is engaged, net cell shape change is rapid. When it is released, net cell shape change proceeds slowly. Amnioserosa data (catch released) courtesy of G. Blanchard.