Pulsation and stabilization: Contractile forces that underlie morphogenesis

Adam C. Martin

Department of Molecular Biology,
Princeton University, Princeton, NJ 08544, USA

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Corresponding author:
Adam C. Martin
am5@princeton.edu
Washington Road
Princeton, NJ 08544, USA

Abstract

Embryonic development involves global changes in tissue shape and architecture that are driven by cell shape changes and rearrangements within cohesive cell sheets. Morphogenetic changes at the cell and tissue-level require that cells generate forces and that these forces are transmitted between the cells of a coherent tissue. Contractile forces generated by the actin-myosin cytoskeleton are critical for morphogenesis, but the cellular and molecular mechanisms of contraction have been elusive for many cell shape changes and movements. Recent studies that have combined live imaging with computational and biophysical approaches have provided new insights into how contractile forces are generated and coordinated between cells and tissues. In this review, we discuss our current understanding of the mechanical forces that shape cells, tissues and embryos, emphasizing the different modes of actomyosin contraction that generate various temporal and spatial patterns of force generation.

Introduction

To see a developing embryo is to see cells in motion. Masses of cells, or tissues, are reshaped into complex but precise three-dimensional structures with unique forms and functions. These macroscopic changes in tissue architecture result from coordinated changes in the shapes and behaviors of individual cells (Gustafson and Wolpert, 1962; Holtfreter, 1943). Cell shape changes (i.e. apical constriction, apical-basal lengthening and shortening, and cell elongation) and cell movements (i.e. intercalation, rotation, and migration) are used ubiquitously through all stages of development in a wide range of species (Friedl and Gilmour, 2009; Lecuit and Lenne, 2007; Leptin, 2005). A major challenge for cell and developmental biologists is to elucidate the cellular mechanisms that drive these cell shape changes and movements and to determine how they collectively deform the tissue.

Networks of actin and myosin II, or the actomyosin cytoskeleton, play a central role in most of the morphogenetic movements that have been studied in model systems (Quintin et al., 2008). The actomyosin cytoskeleton generates contractile or tensile forces in individual cells. These tensile forces can be transmitted between cells of a tissue through cell-cell junctions or through the extracellular matrix. Thus, the actomyosin cytoskeleton is well suited to translate individual cellular behaviors into global changes in tissue mechanics and/or shape. Although the importance of actin and myosin II has been clearly demonstrated, the mechanisms by which actomyosin networks drive cell shape changes and movements are still poorly understood. The subcellular organization and dynamics of actomyosin networks have not been well characterized in developing embryos. In addition, although actomyosin networks presumably generate contractile forces, measurements of these forces have been complicated by the fragility of embryonic tissues and their accessibility to mechanical manipulation. Finally, it is not clear how

contractile forces are coordinated between the cells of a tissue to drive coherent changes in tissue morphology. Elucidating mechanisms of actomyosin contractility and force-generation at the cellular level is critical to understand the tissue-level mechanics of morphogenesis.

Recent quantitative studies have provided insight into the cellular mechanisms of actomyosin contractility during morphogenesis (Supplemental Figure 1). Live imaging of cells and the cytoskeleton, coupled with computational image analysis, has elucidated the organization and dynamics of actomyosin networks as well as the coordination of the resulting cell shape changes and movements in the tissue. In addition, approaches developed to directly measure tensile forces in cells and tissues have revealed the directionality and relative magnitudes of forces generated by contractile networks. This review will focus on the distinct modes of actomyosin contraction that have been observed to function during morphogenesis. First, we will review the molecular mechanisms of how actomyosin generates contractile force and how these forces are transmitted within and between cells. Second, we will analyze the different mechanisms by which actomyosin contractility promotes morphogenesis, using apical constriction, cell intercalation, and cell sheet extension as case studies.

Force generating apparatus: Actomyosin networks and adherens junctions

Engine of morphogenesis: The actomyosin cytoskeleton

The actomyosin cytoskeleton consists of networks of fiber-like actin filaments that are cross-linked by the molecular motor myosin II. Myosin II is a hexamer that consists of two myosin heavy chains (MHC), two regulatory light chains (RLC), and two essential light chains (ELC). MHC contains an amino terminal motor domain that uses the free-energy of ATP hydrolysis to move towards the barbed or (+) end of the actin filament, thus moving the filament in the direction of the pointed or (-) end. The fraction of the time that an individual myosin II head spends attached to a filament, also called the duty ratio, is low (~ 0.1) (Howard, 2001). Thus, processive filament movement requires that 10-30 myosin II hexamers oligomerize tail-to-tail to form bipolar minifilaments, which resemble dumbbells with the motor heads pointed away from each other at the poles of the rod (Verkhovsky and Borisy, 1993). The bipolar nature of myosin II minifilaments allows myosin II to pull actin filaments with antiparallel orientation towards one another, generating contractile, or tensile, force (Figure 1A-C).

The organization of actin and myosin II on the cell cortex influences the nature of the applied cellular force. Several different modes of actomyosin contraction have been observed in non-muscle cells. First, actomyosin networks can have actin and myosin II filaments aligned in a bundle, which we will call an actomyosin cable. Actomyosin cables can adopt the repeated organization of a muscle sarcomere, in which bipolar myosin II minifilaments associate with tandem arrays of antiparallel actin filaments (Cramer et al., 1997; Langanger et al., 1986) (Figure 1A). Myosin II generates contractile force by sliding the antiparallel actin filament arrays towards each other, which shortens the sarcomeric repeat unit. The sarcomeric organization of actomyosin cables is recognized by the alternating pattern of myosin II and the actin filament

cross-linker α-actinin, and is observed in certain types of stress fibers and in circumferential actin cables in some epithelial cells (Hildebrand, 2005; Lazarides and Burridge, 1975; Pellegrin and Mellor, 2007; Weber and Groeschel-Stewart, 1974). Contractile actin cables can also consist of actin filaments with no apparent repeated organization of filament polarity, although the exact mechanism of contraction is not clear (Cramer et al., 1997; Kamasaki et al., 2007; Tanaka-Takiguchi et al., 2004) (Figure 1B). Because of the orientation of actin and myosin II, contractile force is aligned in the direction of contractile cables, generating tensile force between two points or around the cell circumference. In contrast to actin cables, actin and myosin II filaments can be organized into two-dimensional networks in which actin filaments are interwoven in a mesh, which we will call an actomyosin meshwork (Backouche et al., 2006; Bendix et al., 2008; Svitkina et al., 1997; Verkhovsky et al., 1995; Verkhovsky et al., 1997) (Figure 1C). Actomyosin meshworks can contract, decreasing the surface area of the network. Thus, contraction of the actin filament meshworks that underlie the plasma membrane can generate planar tensile forces and cortical surface tension.

Many cells contain both a cortical actomyosin meshwork and linear actin cables. Thus, these different contractile modes or modules can be combined to simultaneously generate different component forces and/or to generate more complicated temporal or spatial patterns of contraction. We will discuss how different contractile modules are combined during morphogenesis later in this review.

Cellular anchors: Adherens junctions

In order for actomyosin contractility to apply force within a cell or transmit forces between cells, actomyosin networks must be mechanically coupled to the cell surface and

between adjacent cells of a tissue. Although mechanical forces can be transmitted between cells indirectly via the extracellular matrix, most of the case studies we examine show evidence of direct cell-cell linkages. In epithelial cells, this mechanical linkage occurs primarily at apical adherens junctions (Gates and Peifer, 2005; Halbleib and Nelson, 2006). Adherens junctions contain the cell surface glycoprotein E-cadherin, whose extracellular domain mediates homophilic cell-cell adhesion. The intracellular domain of E-cadherin forms a complex with β-catenin and the actin-binding protein α-catenin (Ozawa and Kemler, 1992). Although the nature of the connection between E-cadherin and the actin cytoskeleton is unclear (Drees et al., 2005; Yamada et al., 2005), genetic studies suggest that β-catenin and α-catenin help mediate the linkage of actin networks to adherens junctions, and thus the mechanical linkage of neighboring cells (Cavey et al., 2008; Dawes-Hoang et al., 2005; Gates and Peifer, 2005; Vasioukhin and Fuchs, 2001).

Similar to actomyosin contraction, there are different modes by which adherens junctions integrate the actomyosin cytoskeleton between cells. Mature junctions exhibit a continuous E-cadherin band around the apical circumference of the cell, which is associated with a circumferential belt or cable of predominantly unbranched actin filaments oriented parallel to the plane of the junctions (Baker and Schroeder, 1967; Burnside, 1971; Hirokawa et al., 1983; Yonemura et al., 1995). Thus, in mature junctions, cell-cell adhesion is relatively continuous and tension from the actomyosin cytoskeleton is oriented along cellular interfaces, similar to the distribution of tensile forces in two-dimensional foams (Figure 1D). In contrast, nascent cell-cell junctions consist of discrete puncta or spots (Adams et al., 1996; Vasioukhin et al., 2000; Yonemura et al., 1995). Spot junctions associate with radial actin cables that are oriented perpendicular to the plane of the junctions (Figure 1E). Importantly, radial actin cables can form

an intercellular meshwork that spans the apical surface (Vaezi et al., 2002). Integration of actomyosin contractility through spot junctions creates a continuum of tensile force across the cell sheet that is not restricted to cell interfaces. Thus, both the mode of contraction and the nature of the mechanical connections between cellular actomyosin networks have important implications for the mechanical properties of the tissue.

In addition to transmitting contractile forces from actomyosin networks, the preference of cells to maximize their adhesive interactions can generate tissue surface tension (Lecuit and Lenne, 2007; Steinberg, 2007). Tissue surface tension might explain several morphogenetic behaviors, such as the spreading of one tissue over another and the sorting of cells with different affinities into immiscible layers. In the following case studies we examine the cellular mechanisms by which both actomyosin and adhesion generate tension in embryonic tissues and how this results in morphogenesis.

Apical constriction: Purse-strings and ratchets

Epithelial cells are polarized such that there are distinct apical and basal-lateral domains that are separated by adherens junctions (Figure 2A). A common cell shape change of epithelial morphogenesis is apical constriction, which results in a reduction of the apical domain of the cell (Sawyer et al., 2009b). Apical constriction of columnar epithelial cells converts these cells to a cone or wedge shape (Figure 2B). This facilitates the bending or folding of the epithelial sheet (Odell et al., 1981). Thus, apical constriction can be used to generate a variety of epithelial topologies, including folds, pits, and tubes (Colas and Schoenwolf, 2001; Leptin, 2005). Apical constriction is likely to require contractile forces generated by the actomyosin cytoskeleton,

since to constrict the apical cortex of a cell in an integrated cell sheet, the surrounding tissue must be pulled inwards (Figure 2C). How do actin and myosin II generate this contractile force?

Neural tube closure in vertebrates

During neural tube closure, several populations of cells undergo apical constriction at defined hingepoints to fold the neural plate into a tube (Colas and Schoenwolf, 2001). In these epithelia, actin and myosin II are organized into a circumferential cable that is directly associated with the adherens junction of a cell. Electron microscopy and live imaging of cells undergoing apical constriction during amphibian and chick neurulation demonstrated that this cable shortens and increases in thickness upon constriction of the cell apex (Baker and Schroeder, 1967; Burnside, 1971; Ferreira and Hilfer, 1993; Karfunkel, 1972). Treatment of similar circumferential actin cables, either semi-purified or in glycerinated epithelia, with Mg-ATP, which would promote myosin II-dependent actin filament movement, resulted in their constriction (Hirokawa et al., 1983; Owaribe et al., 1981; Owaribe and Masuda, 1982). This led to the model that myosin II induced actin filament sliding contracts the circumferential actin cable, constricting the cell apex like a purse-string (Figure 2D).

Shroom, an actin-binding protein that is localized to adherens junctions and is required for neural tube closure in both mice and *Xenopus* (Haigo et al., 2003; Hildebrand and Soriano, 1999; Nishimura and Takeichi, 2008), possibly organizes actin and myosin II into a contractile purse-string. Shroom is required for apical constriction of neural cells during neural tube formation and ectopic expression of Shroom is sufficient to induce apical constriction (Haigo et al., 2003; Hildebrand, 2005). Importantly, Shroom expression in MDCK cells recruits myosin II and the actin crosslinker α-actinin to adherens junctions, where they adopt a complementary

sarcomeric repeat pattern around the circumference of the cell apex (Hildebrand, 2005). In agreement with the contractile organization of myosin II and α-actinin, interfacial tension appears to increase around the apical circumference of the cell. Although the repeated pattern of myosin II and α-actinin has not been observed in neural epithelia, Shroom mediates myosin II enrichment at apical junctions, possibly by recruiting Rho kinase to junctions, which activates myosin II by phosphorylation (Hildebrand, 2005; Nishimura and Takeichi, 2008). This evidence further supports the purse-string model for apical constriction of neural epithelia, where Shroom possibly organizes junctional actin and myosin II into sarcomere-like circumferential cables.

Mesoderm invagination in Drosophila

During *Drosophila* gastrulation, apical constriction is important for the internalization of the presumptive mesoderm (Costa et al., 1994; Kam et al., 1991; Parks and Wieschaus, 1991; Sweeton et al., 1991). In contrast to the purse-string model, which requires a circumferential actomyosin cable, myosin II staining in mesoderm cells is dispersed across the entire apical cortex (Dawes-Hoang et al., 2005; Young et al., 1991). Recent live imaging and computational image analysis of both apical cell shape and myosin II revealed a novel mechanism for apical constriction (Martin et al., 2009). Apical constriction is correlated with bursts of myosin II coalescence, in which myosin II spots increase in intensity and move together in the plane of the cortex to form larger more intense myosin II structures in the middle of the apical cortex. Myosin coalescence requires the presence of an intact apical actin meshwork, suggesting that it represents actin meshwork contraction. In addition, myosin II coalescence is accompanied by inward folding of the cell surface at E-cadherin spots around the cell circumference, suggesting that meshwork contraction in the middle of the apical cortex pulls the cell surface at discrete

junction attachment sites. This is consistent with phenotypes of mutants that disrupt adherens junctions, where myosin II contracts into a ball in the middle of the apical cortex, presumably because the actin meshwork fails to establish strong attachments to junctions (Dawes-Hoang et al., 2005; Sawyer et al., 2009a). Thus, contraction of a cortical actomyosin meshwork spanning the apical surface, not a circumferential actin belt, drives apical constriction in the *Drosophila* mesoderm (Figure 2E).

Surprisingly, rather than being continuous, apical constriction of the *Drosophila* mesoderm is pulsed, with periods of rapid constriction, corresponding to bursts of myosin II coalescence, interrupted by periods of little or no constriction (Martin et al., 2009). Pulses of constriction are asynchronous and appear to occur in a stochastic pattern across the tissue. Previous studies had suggested that a subpopulation of ventral cells stochastically initiate constriction before the synchronous constriction of the remaining cells (Kam et al., 1991; Sweeton et al., 1991). Live imaging of apical constriction has not clearly identified a discrete subpopulation of cells that initiate constriction first (Martin et al., 2009; Oda and Tsukita, 2001). However, because a framework has been developed to quantify constriction in this system, future statistical analysis will be possible to determine whether there are temporal or spatial patterns to constriction pulses in the tissue. Importantly, between constriction pulses the constricted state of the cell is maintained or stabilized leading to the net constriction of cell apices (Figure 2F). Thus, apical constriction proceeds incrementally via a ratchet-like mechanism of contraction and stabilization (Martin et al., 2009).

What causes the pulses of actomyosin meshwork contraction that power constriction? One possibility is that pulsation results from mechanical interactions between cells. For example, if cell stretching triggers contraction (Odell et al., 1981), contraction of one cell could

induce a contraction pulse in an adjacent cell and vice versa. However, contraction pulses are observed even when mesoderm cells have lost adhesion with one another (Martin and Wieschaus, unpublished data). In addition, periodic myosin contractions are observed in the single cell *C. elegans* embryo and in anuclear cell fragments treated with Nocadazole (Munro et al., 2004; Paluch et al., 2005). Thus, pulsed contractions might not require mechanical cell-cell interactions, but could reflect a dynamic property of cellular actomyosin networks. For example, oscillating contractions could result from cycles of actin filament turnover or myosin II activation/inactivation. In the mesoderm, the transcriptional repressor Snail is required to initiate pulsed contractions (Martin et al., 2009), although the relevant transcriptional targets of Snail are not known. Identification of Snail targets that influence actomyosin contractility could provide insights into the mechanism of pulsed contraction.

It is unclear whether the cortical actomyosin meshwork that powers contraction also stabilizes cell shape between contractions. After contraction pulses, there is significant remodeling of the apical myosin II. However, actomyosin structures, such as fibers, remain on the apical cortex between contraction pulses and could serve as a "catch" to maintain constricted cell shape. The transcription factor Twist is required to stabilize cell shape (Martin et al., 2009). Twist could promote the formation or stabilization of actomyosin fibers by activating G-protein coupled receptor signaling, which activates stress fiber formation in cultured cells (Buhl et al., 1995; Lin et al., 2005). Twist could also influence cortical tension and thus cell stabilization through its requirement for the assembly of apical junctions (Kölsch et al., 2007). Analysis of actomyosin and adherens junctions in mutants that affect downstream components of the Twist pathway, such as Fog, T48, and Rho kinase, is required to elucidate the mechanism of stabilization.

Comparing apical constriction in the neural tube to that of the *Drosophila* mesoderm reveals that different modes of actomyosin contractility are used to drive similar cell shape changes. Purse-strings and meshworks both appear to be conserved in development. Many epithelia contain a circumferential actomyosin belt and tension generated by this belt is required to maintain ordered cell shape as well as drive apical constriction (Farhadifar et al., 2007). However, myosin II is localized across the apical surface during other apical constriction events, including *C. elegans* and *Xenopus* gastrulation, suggesting a mechanism similar to meshwork contraction (Lee and Goldstein, 2003; Lee and Harland, 2007; Nance and Priess, 2002). It is likely that there are mechanical reasons for utilizing distinct cytoskeletal mechanisms for contraction. Because an actomyosin purse-string must generate tensile force all around the cell circumference to constrict the cell, purse-strings might be most effective at generating isotropic forces. In contrast, cortical actomyosin meshworks could generate anisotropic tensile forces that constrict the cell because they bridge the apical surface. In addition, the pulsatile behavior of the actomyosin meshwork might help coordinate cell behavior in the tissue. If apical constriction occurred in a single step, then the cells that stochastically initiated constriction first could irreversibly stretch their neighbors. Taking several small steps to achieve a final constricted state might allow feedback mechanisms to balance contractile forces across the tissue. Indeed, in addition to future endodermal cells showing a transcriptional response to forces developed by morphogenetic movements at gastrulation (Desprat et al., 2008; Farge, 2003), mesoderm cells exhibit actomyosin contractility in response to mechanical stimuli that could mediate contractile force propagation across the mesoderm (Pouille et al., 2009). Thus, incremental cell shape changes driven by pulsatile contractile networks could allow developmental plasticity that allows cells to adapt and balance forces across a large tissue. Future experiments that measure forces

generated by purse-strings and meshworks during apical constriction are required to determine how these mechanisms influence tissue mechanics.

Cell intercalation: Anisotropic actomyosin cables and dynamic actin meshworks

During gastrulation and neurulation, convergence (narrowing) and extension (lengthening) elongates the anterior-posterior body axis of the embryo. Convergence and extension can be driven by polarized mediolateral or radial cell-cell intercalations, directed cell migration, cell shape change, and directed cell division. We focus here on the mechanisms of polarized cell-cell intercalation, the process by which cells move between each other to change the aspect ratio of the tissue (Keller et al., 2008; Lecuit and Lenne, 2007). Contractile forces could pull originally non-neighboring cells together. In addition, cells may need to resist compression, or exhibit stiffness, as they wedge between one another and push the tissue along the axis of extension. Alternatively, cell intercalation could be a passive response to external tensile forces that stretch the tissue along the axis of extension. Do actin and myosin II generated forces drive cell-cell intercalation and by what mechanisms do they generate this force?

Germband extension in Drosophila

During *Drosophila* gastrulation, stereotypic cell rearrangements in the plane of the epithelium appear to be responsible for the convergence and extension that drives germband extension (Irvine and Wieschaus, 1994). Interfaces oriented parallel to the dorsal-ventral axis of the embryo, which separate anterior and posterior cell neighbors (A-P interfaces or type 1 junctions) preferentially contract to form a type 2 junction in which four cells meet at a common

vertex (Bertet et al., 2004) (Figure 3A). Type 2 junctions directionally resolve to form a new interface between dorsal and ventral cells that were previously separated (D-V interfaces or type 3 junctions). In addition, higher order intercalation events involving several aligned A-P interfaces form intermediate rosette structures in which 5-11 cells meet at a common vertex, which resolve into multiple new D-V interfaces (Blankenship et al., 2006) (Figure 3B). Both types of rearrangements appear to contribute to the 2.5 – 3-fold elongation of the germband.

What drives the initial contraction of A-P interfaces? During cell intercalation, F-actin and myosin II become enriched at A-P interfaces, forming what appear to be actomyosin cables underlying the adherens junctions (Bertet et al., 2004; Blankenship et al., 2006; Zallen and Wieschaus, 2004) (Figure 3D). In addition, myosin II activity is required for the cell intercalation events in the germband (Bertet et al., 2004). In forming rosettes, actomyosin cables are aligned across multiple A-P interfaces (Blankenship et al., 2006) (Figure 3E). Interestingly, A-P interfaces in forming rosettes take longer to contract and undergo rounds of contraction and extension, suggesting that actomyosin cables generate tensile forces that dynamically resist each other during contraction. These observations led to a model in which actomyosin cables generate anisotropic tension around the cell circumference, resulting in higher tension at A-P interfaces causing them to contract (Figure 3D and E). Recent laser cutting experiments have tested this model by comparing tensile forces in A-P interfaces, which are enriched in actomyosin, to D-V interfaces (Rauzi et al., 2008). This study demonstrated that A-P interfaces have ~ 1.8-fold higher tension than D-V interfaces, a value sufficient to drive tissue elongation in a mathematical simulation of germband extension.

While anisotropic tension is likely to contract the A-P interface, it is not clear how contraction of the actomyosin cable is coupled to the removal of adherens junctions from the

contracting interface. If the connection of the actin cytoskeleton to adherens junctions is dynamic, as was recently proposed (Drees et al., 2005; Yamada et al., 2005), it is possible that constitutive adherens junction turnover can accompany the contraction of the A-P interface. However, *Drosophila* epithelia have adherens junction microdomains that can be stable for over an hour (Cavey et al., 2008), which is much longer than the ~ 10 minutes that it takes for A-P interface contraction. Thus, germband cells might need a mechanism to actively destabilize and remove junctions underlying actomyosin cables. This contrasts with other systems where actomyosin contractility strengthens cell-cell adhesion (Conti and Adelstein, 2008).

Interestingly, laser cutting of actin cables causes contraction of the cable along a single interface, stopping at the cell vertices (Cavey et al., 2008; Rauzi et al., 2008). Thus, rather than the actomyosin network being a continuous structure around the cell circumference, actin cables are mechanically compartmentalized to discrete interfaces. In addition, interfacial actin cables appear to be stably anchored at vertices. This anchoring at vertices could explain how actin cables become aligned across multiple cells in forming rosettes (Blankenship et al., 2006). The compartmentalization of cortical actomyosin networks could establish domains that can be differentially regulated to generate anisotropic tension around the cell circumference.

Contraction of A-P interfaces only extends the tissue halfway. A substantial component of extension results from the resolution of a type 2 junction or rosette and the expansion of new cell-cell interfaces (Figure 3A and B). Resolution is associated with disassembly of actin and myosin II concentrated at the multicellular vertex of type 2 junctions and rosettes and the appearance of a new E-cadherin-containing interface that subsequently incorporates Bazooka/Par-3, which could stabilize junctions (Blankenship et al., 2006). Does this interface grow against compressive forces that resist extension of the tissue? A recent study that mapped

strain rates in germband cells suggests that rather than undergoing compression, germband cells are stretched by an external tensile force directed along the axis of extension (Butler et al., 2009). Thus, the expansion of D-V interfaces may be a passive response of the tissue. A similar mechanism to relieve tensile stress was also shown for the intercalation of *Drosophila* trachea cells (Caussinus et al., 2008). Interestingly, the external tensile stress appears to result from mesoderm invagination (Butler et al., 2009), demonstrating how distinct morphogenetic movements are linked to each other in a mechanically integrated system.

Convergence and extension in vertebrates

In vertebrate development, convergence and extension in dorsal tissues, such as the dorsal mesoderm and neural ectoderm, extend the embryonic axis during gastrulation and neurulation. Experimental approaches that have measured the forces associated with convergence and extension in *Xenopus* have determined mechanical properties of the tissue during this process. Dorsal explants of tissue undergo convergence and extension in culture and can produce up to 1.2 μN of pushing force before buckling (Keller and Danilchik, 1988; Moore, 1994). Thus, convergence and extension is driven by forces generated autonomously within the dorsal tissue and not by external forces. To generate this pushing force, the tissue must provide mechanical support to maintain a straight body axis, even as the cross-sectional area of the tissue decreases due to convergence. The stiffness, or resistance to compression or bending, of dorsal explants increases along the axis of extension as dorsal tissues undergo convergence and extension during gastrulation and neurulation (Moore et al., 1995; Zhou et al., 2009). This increase in stiffness is tissue specific, with certain tissues providing the majority of the mechanical support along the axis of lengthening (Zhou et al., 2009). Thus, tissue stiffness is

tightly regulated in both time and space and apparently compensates for the narrowing of the tissue. Actin and myosin II are both required for the high level of stiffness in dorsal explants (Rolo et al., 2009; Zhou et al., 2009). Therefore, examining the cellular mechanisms of contraction and cell movement will be important to understand the basis of the changes in tissue mechanics.

In contrast to the planar rearrangement of epithelial cell-cell contacts that occurs during germband extension, vertebrate convergence and extension is driven by the polarized migration and rearrangement of mesenchymal cells. Live imaging of explants and embryos has demonstrated that convergence and extension occurs both by radial and mediolateral intercalation of cells, occurring preferentially between neighbors along the axis of extension (Keller et al., 2008; Yin et al., 2008). Mediolateral intercalation appears to be driven by mediolaterally polarized protrusions that episodically extend and retract from the cell (Shih and Keller, 1992). These protrusions appear to allow cells to exert tension on each other such that cells become elongated along the mediolateral direction. These elongated cells then wedge between one another to push the tissue along the axis of extension (Figure 3C).

Live imaging of the actin cytoskeleton in *Xenopus* cells undergoing mediolateral intercalation showed that both mesoderm and neural ectoderm cells assemble a network of actin filament cables that emanate from actin dense foci (Rolo et al., 2009; Skoglund et al., 2008) (Figure 3F). Importantly, these actin networks are dynamic and appear to undergo myosin-dependent episodic or pulsed contractions that are oriented along the axis of intercalation, the long axis of the cells (Rolo et al., 2009; Skoglund et al., 2008). Contractions along the long axis could generate tissue stiffness by preventing cells from being compressed along the axis of extension. The pulsatile nature of contraction could provide a mechanistic basis for the

repetitive tugging and incremental cell intercalation that were described in a recent review (Keller and Shook, 2008). The dynamics of cell body movement and the actin cytoskeleton need to be quantified to definitively demonstrate that contractile forces generated by the actin cytoskeleton are episodic and are correlated with incremental movements of cells between one another. In addition, it will be important to establish whether the actin network exerts tension directly on neighboring cells or whether this occurs indirectly through the extracellular matrix. However, cytoskeletal dynamics in these cells parallel those that occur in the apical meshwork of the *Drosophila* mesoderm, suggesting that pulsatile contraction may be a general feature of actomyosin meshworks that function during morphogenesis.

Epiboly and cell sheet extension: Tissue surface tension and supracellular actin cables

The spreading or extension of a tissue or cell sheet to close an opening is a common morphogenetic movement in both development and tissue repair. Cell sheet extension often involves cell shape changes and rearrangements in multiple tissues, including cell intercalation, cell elongation, and cell constriction (Martin and Parkhurst, 2004). Forces that could contribute to cell sheet extension include differences in tissue surface tension that allow one cell type to envelope another, pulling of an epithelial margin by a neighboring tissue, the purse-string-like contraction of marginal cells to reduce their perimeter, or active migration of cells that push the margin forward. Analysis of cell and cytoskeletal dynamics during this process suggests that actomyosin contraction and adhesion play important roles in moving epithelial sheets forward. We again find that different modes of contractility drive similar movements in different tissues.

Zebrafish epiboly and gastrulation

During zebrafish gastrulation, several spreading events establish the body plan of the embryo. First, blastoderm cells piled on the animal pole of the yolk cell undergo thinning and extension towards the vegetal pole of the embryo, a process called epiboly (Solnica-Krezel, 2006). This spreading involves both a superficial, squamous epithelia called the enveloping layer (EVL), more mesenchymal deep cells, which consist of both ectoderm and mesendoderm (mesoderm and endoderm), and a syncytial layer of yolk nuclei (yolk syncytial layer, YSL) (Figure 4A). At 50 % epiboly, the more vegetally located mesendoderm cells segregate from and migrate underneath the overlying ectoderm in the direction of the animal pole (Montero et al., 2005). Despite their mesenchymal nature, migrating ectoderm and mesendoderm fail to intermix as they migrate in opposite directions past one another, suggesting that mechanical forces stabilize these layers.

Cell spreading and sorting behaviors can be explained by differential surface tension between groups of cells (Steinberg, 1963). Tissue surface tension results from tensile forces that act to minimize the surface area of a tissue (Lecuit and Lenne, 2007). Measurements of aggregate surface tension and cell sorting assays have demonstrated that dissociated cells from zebrafish, or other embryos, separate into different layers, with cell types having lower surface tension enveloping those with higher surface tension (Foty et al., 1996; Foty and Steinberg, 2005; Schötz et al., 2008). However, in zebrafish and amphibian embryos, even though ectoderm cells exhibit the highest surface tension and adopt the innermost position in cell sorting assays, the ectoderm remains the outermost germ layer *in vivo* (Phillips and Davis, 1978; Schötz et al., 2008). This inversion results from the coating of germ layers by a polarized epithelial layer (EVL in zebrafish) with a strongly adhesive inner side and a “non-adhesive” outer side (Ninomiya and Winklbauer, 2008; Phillips and Davis, 1978). Adhesive interactions between the

EVL and the ectoderm and the resulting surface tension effects could stabilize ectoderm cells in the outermost germ layer, allowing mesendoderm cells to migrate underneath the ectoderm as a separate, immiscible layer.

What is the molecular basis of tissue surface tension? Similar to surface tension in liquids, where molecules maximize their collective interactions by minimizing global surface area, tissue surface tension could result from cells maximizing adhesive interactions (Steinberg, 1963). In this case, cell sorting and spreading behaviors can be driven by differences in adhesive energies between different cell types. This differential adhesion hypothesis is supported by experiments that show that tissue surface tension and cell sorting behaviors correlate with cadherin expression (Foty and Steinberg, 2005). Thus, cell types with the highest surface tension, such as the zebrafish ectoderm, are expected to have the highest adhesion. However, a recent study that used several methods to measure cell-cell adhesion found that the ectoderm has the lowest adhesive energy of all germ layers (Krieg et al., 2008). Instead, tissue surface tension was correlated with cell contractility (Krieg et al., 2008), which supports models that suggest that differential surface contraction, resulting from actomyosin contractility, is critical for tissue surface tension and cell sorting (Brodland, 2002; Harris, 1976). Disruption of actomyosin contractility did disrupt germ layer cell sorting (Krieg et al., 2008), although it is important to consider that contractility likely affects cell-cell adhesion and vice versa (Conti and Adelstein, 2008). Despite limitations in functionally separating adhesion and contraction, these studies highlight the complex interplay between these cell properties, which both influence tissue surface tension and mechanics.

Experiments in intact zebrafish embryos support a role for adhesion in cell spreading. E-cadherin expression is required both for deep cell epiboly and for anterior mesendoderm

migration underneath the ectoderm (Kane et al., 2005; Montero et al., 2005; Shimizu et al., 2005; Ulrich et al., 2005). Importantly, a radial gradient of E-cadherin mRNA expression is observed in which E-cadherin expression increases towards the embryo surface, being highest in the outermost EVL and the ectoderm (Kane et al., 2005). These results are consistent with a model in which deep cell spreading and sorting results from cells maximizing adhesive interactions with each other and the EVL, but contradicts measurements of cadherin protein expression and adhesive energy in dissociated ectoderm cells, which showed that ectoderm cells have the lowest levels of adhesion (Krieg et al., 2008). This discrepancy must be resolved in order to determine the mechanism by which adhesion promotes cell movements. In addition, it will be important to examine actomyosin dynamics and the function of contractility in both ectoderm and mesendoderm cell migration in live embryos.

In contrast to deep cell epiboly and gastrulation movements, EVL epiboly is not inhibited by E-cadherin mutants, suggesting that an independent mechanism drives this movement (Kane et al., 2005; Lin et al., 2009). EVL cells become elongated in the direction of extension, suggesting that contractile forces could pull this tissue vegetally. Consistent with a vegetal pulling force, epiboly is associated with actin rings that encircle the yolk cell at the margin of the tissue (Cheng et al., 2004). The most prominent of these rings is a wide (~ 16 μm), punctate actin band that appears in the yolk cell as the EVL margin reaches the equator (Figure 4A). Over the course of epiboly, this ring contracts along its width and increases in intensity to create a continuous actin purse-string that encircles the yolk cell immediately vegetal to the EVL (Figure 4A). This possibly represents contraction of an actin meshwork since myosin II also localizes to puncta in the ring and is required for the increase in actin intensity (Koppen, 2006). Importantly, the appearance of this actomyosin purse-string is associated with cell shape changes in marginal

EVL cells that suggest the generation of tension (Koppen, 2006). This includes the straightening of the marginal edge, elongation of the cells in the direction of extension, and constriction of EVL cell interfaces that border the purse-string. Tension in the yolk actomyosin purse-string appears to be transmitted to EVL cells by tight junctions that link the EVL and the yolk cell (Betchaku and Trinkaus, 1978; Koppen, 2006). Thus, EVL epiboly appears to be driven by the purse-string-like contraction of an actomyosin band that encircles the yolk cell. Future experiments are required to examine if there is tension present in the EVL layer or the purse string and to examine actin and myosin II dynamics during actin purse-string contraction.

Dorsal closure

Dorsal closure during *Drosophila* development closes the dorsal opening in the epidermis of the embryo (Figure 4B). Like epiboly, dorsal closure involves spreading of an epithelial sheet across the underlying tissue. Multiple cellular processes contribute to dorsal closure, including: 1) apical constriction of a squamous epithelium, the amnioserosa, that occupies the dorsal hole in the epidermis (Gorfinkiel et al., 2009; Kiehart et al., 2000); 2) contraction of a supracellular actomyosin purse-string in the leading-edge of the epidermis that surrounds the amnioserosa (Kiehart et al., 2000; Young et al., 1991); 3) filopodial extension and zipping of the lateral epidermal sheets as they meet along the dorsal midline (Jacinto et al., 2000); 4) apoptosis and extrusion of amnioserosa cells (Toyama et al., 2008). Laser cutting experiments revealed that contractile forces from the amnioserosa and the supracellular actomyosin purse-string are the main contributors that pull the margin of the lateral epidermis to the dorsal midline (Hutson, 2003; Kiehart et al., 2000). These contractile forces are mostly balanced by the mechanical resistance in the epidermis. In fact, the combined component forces are 3 orders of magnitude

higher than the net applied force at the leading edge (Hutson et al., 2003; Peralta et al., 2007). Thus, both the amnioserosa and the epidermis experience high levels of tension throughout dorsal closure.

The organization and dynamics of the supracellular actomyosin purse-string in the marginal epidermis suggests that it functions as a tensile cable. At early stages of dorsal closure, marginal epidermal cells are scalloped and unconstricted. Assembly of the actomyosin cable straightens the leading edge and contracts epidermal cells (Jacinto et al., 2002; Solon et al., 2009). Myosin II along the purse-string has a punctate distribution that alternates with α-actinin, suggesting a sarcomeric organization (Rodriguez-Diaz et al., 2008) (Figure 4B). Consistent with a sarcomeric mode of contraction, laser cutting shows that the majority of the contractile force is oriented along the length of the cable, perpendicular to the direction of dorsal closure (Hutson et al., 2003). Despite tension in this purse-string, the average distance between distinct myosin II puncta does not change after the initial straightening of the leading edge (Peralta et al., 2008). However, loss of tension in the epidermal margin in mosaic myosin II mutants leads to stretching of epidermal cells (Franke et al., 2005). Thus, during later stages of dorsal closure, the purse-string at the epidermal margin does not contract, but generates tension that stabilizes cell shape.

In contrast to the balanced tension generated by the supracellular actomyosin purse-string of the epidermis, amnioserosa cells undergo dramatic pulsatile contractions and relaxations as they undergo apical constriction (Solon et al., 2009). Amnioserosa cells have an actin filament meshwork that spans the apical cortex in which bursts of myosin II are observed (Franke et al., 2005) (Figure 4B). Laser incisions in the middle of the apical cortex relieve tension, demonstrating that this actin meshwork forms a tensile sheet that spans the apical cortex of amnioserosa cells (Ma et al., 2009). Cell-cell junctions between amnioserosa have a folded

appearance (Homem and Peifer, 2008), further suggesting that contractile forces generated across the apical cortex pull the cell surface inward during constriction. Thus, amnioserosa apical constriction is similar to that of the *Drosophila* mesoderm, where contraction of an apical meshwork pulls the cell surface inward from the middle of the apical cortex.

Mechanical interactions appear to coordinate contraction pulses in the amnioserosa (Solon et al., 2009). Contraction pulses tend to occur either directly in-phase or out-of-phase with pulses in adjacent cells. In addition, the phase relationship of contractions in neighboring cells can shift, such that cells quickly switch between contracting in-phase and out-of-phase. This behavior can be explained by a mechanical model in which stretching of a cell by its neighbor triggers a delayed autonomous contraction (Solon et al., 2009). Indeed, if tension in the amnioserosa is released by laser cutting, pulsation ceases around the laser incision. In agreement with the model, this could result from failure to stretch cells and to trigger a contraction. However, because wounding induces the rapid formation of additional actomyosin purse-strings (Kiehart et al., 2000; Rodriguez-Diaz et al., 2008), an alternative interpretation is that these actomyosin cables locally stabilize cell shape in adjacent cells. Further dissecting the mechanism by which contractions are triggered would help differentiate these models. One attractive possibility is that cell stretching triggers Ca^{2+} release, which activates myosin II contractility.

Despite dynamic, pulsatile cellular behaviors during *Drosophila* dorsal closure and mesoderm invagination, and *Xenopus* convergence and extension, the resulting global tissue movements are relatively smooth. How do such dynamics result in a continuous movement? One possibility is the cooperation of different actomyosin contractile modes. Over the course of dorsal closure, relaxation of the most peripheral amnioserosa cells dampens and the contracted

cell shape is stabilized. Stabilization of amnioserosa cell shape is correlated with the assembly of a supracellular actomyosin cable. Thus, the supracellular actomyosin cable might function like a ratchet by clamping and gradually decreasing the outer boundary of the amnioserosa (Solon et al., 2009). Thus, the continuous nature of dorsal closure could reflect stabilization of dynamic amnioserosa contractions by the actomyosin purse-string. Interestingly, the supracellular purse-string does not function at the length scale of the entire tissue. Laser cutting experiments demonstrate that disrupting the supracellular actomyosin cable on one side of the embryo does not affect closure on the other side (Rodriguez-Diaz et al., 2008). In addition, tensile forces fall off rapidly away from laser incisions, having little effect at length scales greater than 60 μm (Ma et al., 2009; Rodriguez-Diaz et al., 2008). This suggests that the actomyosin cable locally stabilizes adjacent cells, which propagates into the amnioserosa tissue. Although more work is required to show the mechanism by which the actomyosin cable stabilizes pulsed contractions, dorsal closure clearly illustrates how different contractile modes can be combined to control the dynamics of a morphogenetic movement.

Conclusion

Live imaging and quantitative analysis of cell shape and cytoskeletal dynamics demonstrate that different contractile modes generate unique patterns of force that underlie tissue morphogenesis. In the case studies we have examined, actomyosin meshworks mostly generate dynamic, pulsatile contractile force while more stable contractile forces are generated by actomyosin cables and purse-strings. The type of contractile network used by cells undergoing cell shape changes or movements is likely to influence the overall mechanics of cells and tissues. Pulsatile contractions might be required for cell shape changes or movements that require

actomyosin network remodeling or require mechanical feedback to coordinate incremental cell shape changes. Actomyosin cables could be required to stabilize cell or tissue shape. In cases such as dorsal closure, stable actomyosin cables cooperate with dynamic meshworks to drive continuous changes in tissue morphology. Thus, various contractile modes can be combined in the same cell or in neighboring tissues to generate complex patterns of forces that contribute to development.

Despite, or as a consequence of, these recent advances, many questions remain regarding contractile forces during embryonic development. First, while we have proposed models for actomyosin contraction based on the observed organization and dynamics of actin networks in different systems, they are just that, models. The molecular mechanisms of cytoskeletal contractility must be further explored for each individual cell shape change. Important questions include: Is actin or myosin II filament turnover required to produce force? What is the organization and polarity of actin filaments within these networks? How are different actomyosin networks coupled to neighboring cells or the substrate? What other cytoskeletal proteins are required for contraction? In addition, the continued development of methods to spatially inhibit or disrupt actin or myosin II activity are required to determine the contributions of cortical actin structures in different regions of the cell (i.e. basal-lateral vs. apical) to cell shape change and tissue mechanics (Ma et al., 2009).

Second, cortical contractility must be coordinated with the activities of the microtubule cytoskeleton and other cellular processes, such as membrane trafficking, to effectively change cell shape. During zebrafish epiboly, the advance of blastoderm margin and the yolk cell actomyosin ring is associated with changes in microtubule organization and local endocytosis in the yolk cell (Betchaku and Trinkaus, 1986; Solnica-Krezel and Driever, 1994). This parallels

the situation during *Drosophila* cellularization, where a global actomyosin network associated with the tips of invaginating furrows is associated with local endocytosis and possibly microtubules (Royou et al., 2004; Sokac and Wieschaus, 2008). Understanding how microtubules, membrane trafficking, and other cellular processes regulate actomyosin contractility and vice versa will be necessary to determine how macromolecular machineries cooperate to change cell shape and tissue architecture.

Finally, contractile forces generated by individual cells must be coordinated across the tissue to drive precise rearrangements in tissue architecture. One of the most striking findings from recent studies is how dynamic, pulsatile forces underlie continuous tissue movements. Do mechanical properties such as tension or stiffness rhythmically change in cells or tissues during morphogenetic movements? Do cellular dynamics simply average out or do feedback mechanisms and supracellular cytoskeletal structures "oversee" global tissue movements? With the new approaches that have been developed to measure and analyze cell and cytoskeletal dynamics, we are poised to answer these questions.

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Figure Legends

Figure 1. Modes of actomyosin contraction and cell-cell adhesion. A) Sarcomeric cable contraction. Actin filaments (red) are assembled into arrays with the barbed ends facing away from myosin II minifilaments (green). In regions lacking myosin II, α-actinin (blue) cross-links actin filaments into bundles. The sarcomeric unit is repeated to generate a cable composed of multiple contractile units that can shorten and generate linear tension. B) Mixed polarity cable contraction. Cables of unbranched filaments with little or no organization of filament polarity contract along the length of the cable. C) Meshwork contraction. A two-dimensional actin filament meshwork is contracted by myosin II to generate planar tensile forces. D) Continuous adherens junctions. Adherens junctions form a continuous belt around the cell circumference. These junctions are associated with a circumferential cable of unbranched actin filaments that runs parallel to the junctions and the cell surface. E) Spot adherens junctions. Discrete adherens junction puncta associate with radial actin fibers that are oriented perpendicular to the cell surface and form a tensile sheet across the apical cortex.

Figure 2. Mechanisms of apical constriction. A) Schematic of the polarized organization of an epithelial cell. B) Apical-basal cross-section of cells undergoing apical constriction. Apical constriction facilitates the bending/folding of epithelia by causing cells to adopt a wedge shape. C) Apical surface view of apical constriction. Apical constriction pulls neighboring cells inward, suggesting that contractile forces generate tension in constricting cells. D) The purse-string model of apical constriction. Contractile force generated by myosin II-driven actin filament sliding within the circumferential cable constricts the cell apex. Forces are generated parallel to the cell surface. E) The meshwork model of apical constriction. Myosin II contracts a two-

dimensional actin meshwork that spans the apical cortex of the cell. Forces generated perpendicular to the cell surface pull discrete adherens junction sites inward to constrict the cell. F) Ratchet model of apical constriction. Apical constriction is separated into distinct phases of contraction and stabilization such that the cell constricts incrementally.

Figure 3. Mechanisms of convergence and extension. A) First-order epithelial rearrangements that result from contraction of a single A-P interface resulting in a type 2 junction intermediate of 4 cells. B) Higher-order cell intercalation events that form by the contraction of aligned A-P interfaces, forming a rosette intermediate composed of 5-11 cells. C) Mediolateral cell intercalation of mesenchymal cells during vertebrate convergence and extension. D) Mechanism for first-order intercalation. Anisotropic actomyosin tension generated by an actomyosin cable oriented along A-P interfaces leads to contraction of these interfaces. In addition, adherens junctions are removed via an unknown mechanism. E) Mechanism of higher-order rosette mediated intercalation. Actomyosin cables align between multiple cells to generate supracellular tension that reduces the entire length of the combined interfaces, also displacing junctions. F) Mesenchymal mediated intercalation. Episodic lamelliform extensions and actin network contractions cause cells to wedge between one another.

Figure 4. Mechanisms of cell sheet extension. A) Zebrafish epiboly and gastrulation. Several cell layers, including an outer enveloping layer (EVL), an inner deep cell layer (includes the ectoderm and mesendoderm), and a syncytial layer of yolk nuclei (YSL) extend towards the vegetal pole of the embryo. Epiboly of the deep cells and spreading of the mesendoderm between the ectoderm and the yolk cell could be driven by tissue surface tension. Extension of

the EVL layer is likely driven by contraction of the actomyosin band (red) that encircles the yolk like a purse-string. B) *Drosophila* dorsal closure. A hole in the dorsal surface of the embryo, which is occupied by a squamous epithelia, the amnioserosa, is closed via the dorsal movement of the epidermis. This dorsal movement is driven by pulsed contractions of the amnioserosa and a tensile actomyosin cable at the margin of the epidermis. The actomyosin cable could function like a ratchet to clamp the size of the amnioserosa as these cells constrict.

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Figure 1

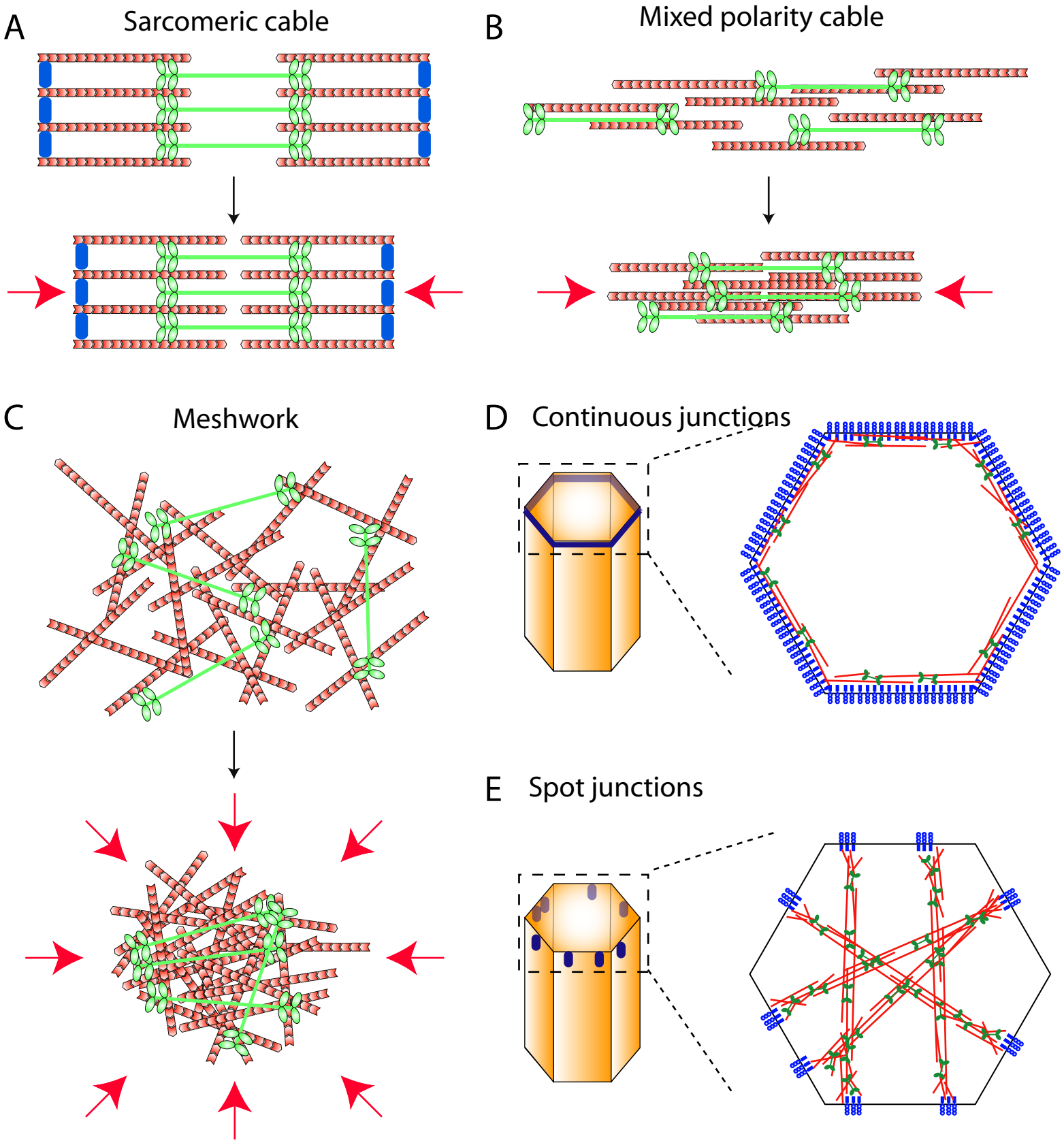

A
Sarcomeric cable
B
Mixed polarity cable
C
Meshwork
D
Continuous junctions
E
Spot junctions

Figure 2

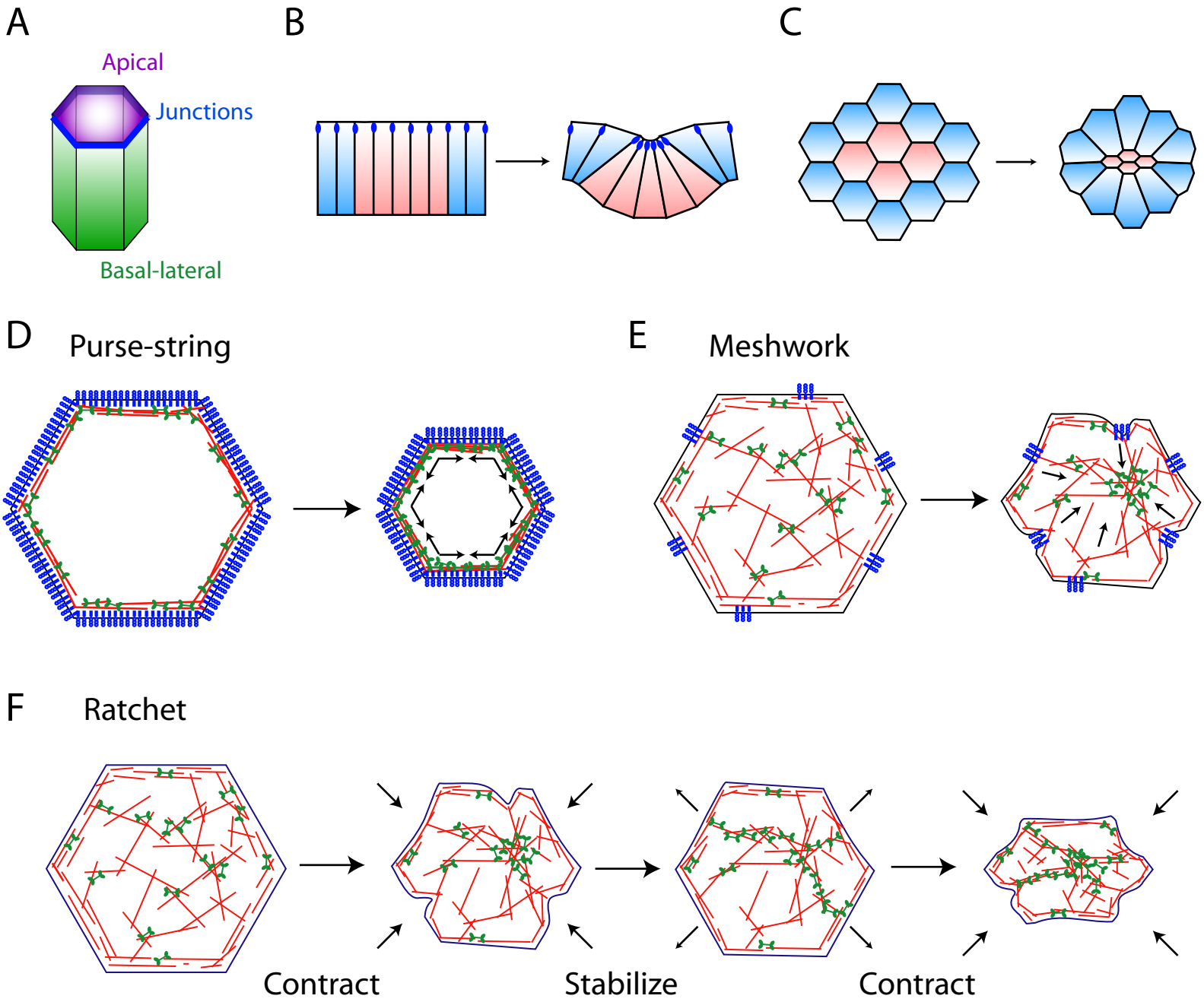

A
Apical
Junctions
Basal-lateral
B
C
D
Purse-string
E
Meshwork
F
Ratchet
Contract
Stabilize
Contract

Figure 3

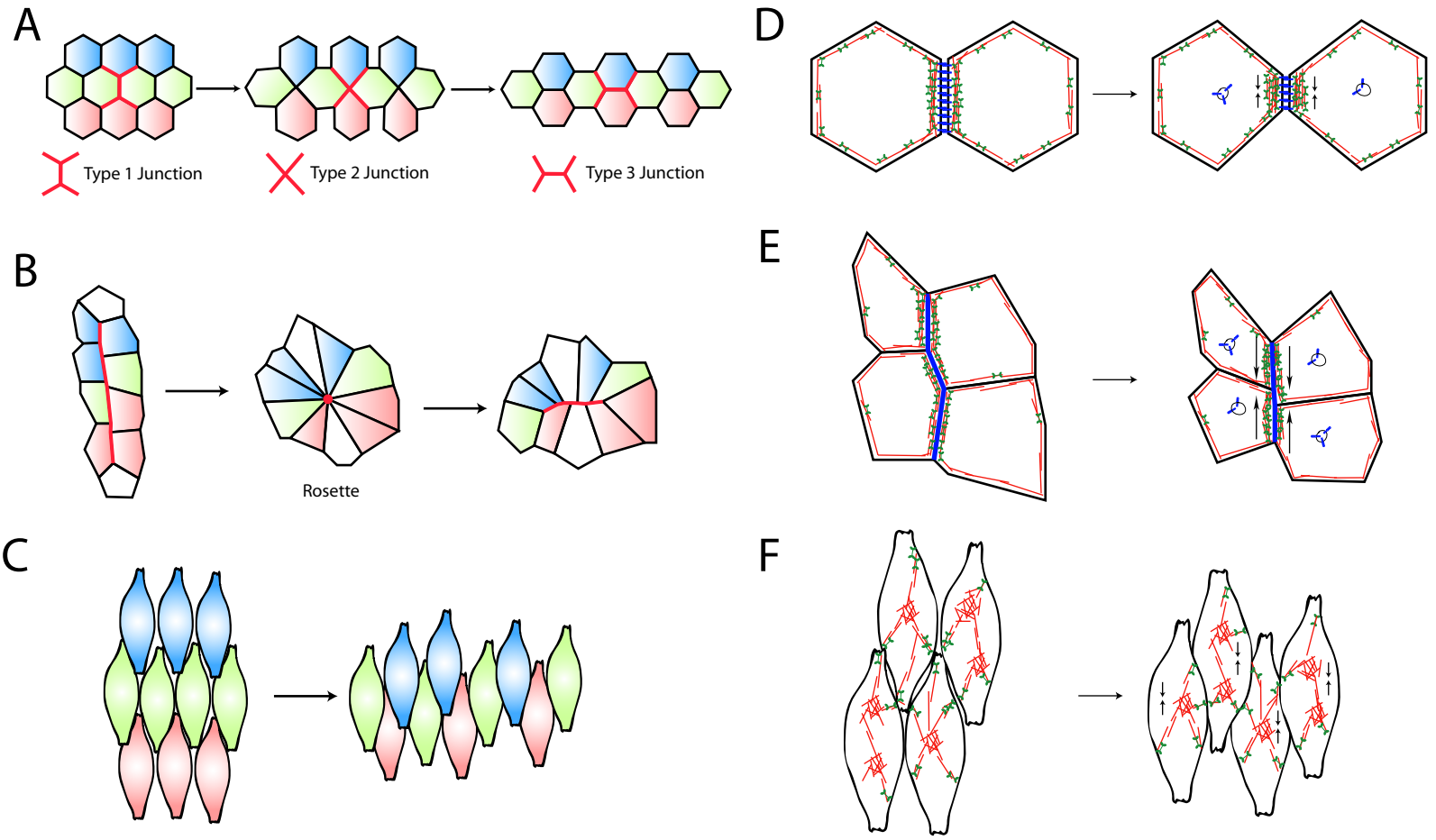
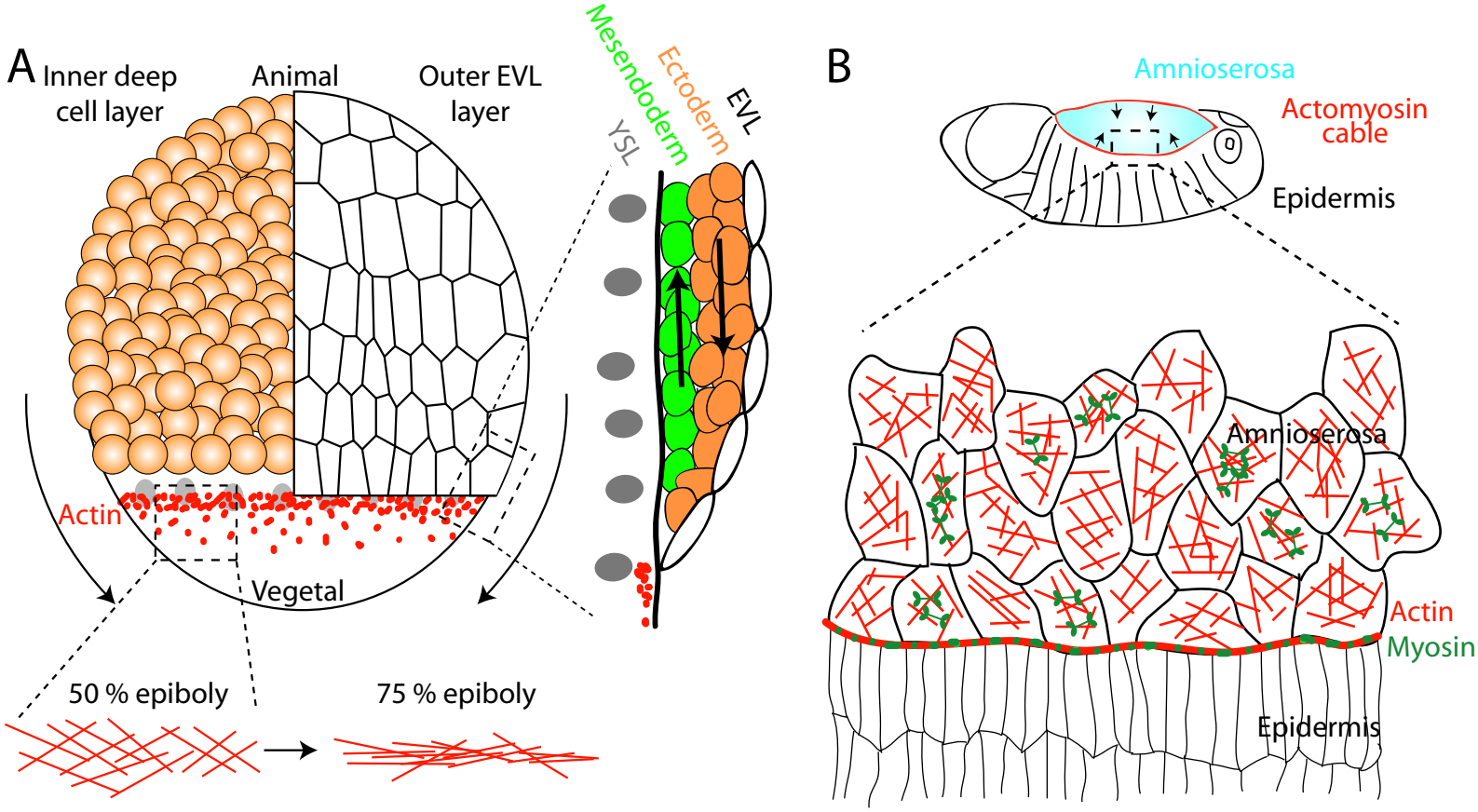

A
Type 1 Junction
Type 2 Junction
Type 3 Junction
B
Rosette
C
D
E
F

Figure 4


A
Inner deep cell layer
Animal
Outer EVL layer
Mesendoderm
Ectoderm
EVL
YSL
Actin
Vegetal
50 % epiboly
75 % epiboly
B
Amnioserosa
Actomyosin cable
Epidermis
Amnioserosa
Actin
Myosin
Epidermis