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REVIEW

Apical constriction: themes and variations on a cellular mechanism driving morphogenesis

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ABSTRACT

Apical constriction is a cell shape change that promotes tissue remodeling in a variety of homeostatic and developmental contexts, including gastrulation in many organisms and neural tube formation in vertebrates. In recent years, progress has been made towards understanding how the distinct cell biological processes that together drive apical constriction are coordinated. These processes include the contraction of actin-myosin networks, which generates force, and the attachment of actin networks to cell-cell junctions, which allows forces to be transmitted between cells. Different cell types regulate contractility and adhesion in unique ways, resulting in apical constriction with varying dynamics and subcellular organizations, as well as a variety of resulting tissue shape changes. Understanding both the common themes and the variations in apical constriction mechanisms promises to provide insight into the mechanics that underlie tissue morphogenesis.

KEY WORDS: Actin, Adhesion, Apical, Cadherin, Constriction, Myosin

Introduction

Epithelial tissues consist of cells that are polarized along their apical-basal axes and held together by cell-cell adhesion. At various times in the life of an organism, epithelial tissues undergo remodeling events, thereby sculpting organs and organisms into different shapes and maintaining tissue homeostasis as cells divide or die. Epithelial remodeling results from dynamic cell shape changes of individual, constituent epithelial cells (Quintin et al., 2008; Heisenberg and Bellaiche, 2013). One such cell shape change is apical constriction, being defined by the shrinkage of the apical side of an epithelial cell (generally the exterior or lumen-facing side of the cell), often causing a columnar or cuboidal cell to become trapezoidal, wedge-shaped or bottle-shaped. The shrinkage of the apical surface of a cell and the resulting change in cell geometry can have different consequences depending on the physiological context (Fig. 1). Apical constriction of populations of cells that maintain cell-cell adhesion can bend and fold epithelial tissues, in some cases transforming flat epithelial sheets into three-dimensional structures, such as tubes (Lewis, 1947; Hardin and Keller, 1988; Alvarez and Navascués, 1990; Kam et al., 1991; Sweeton et al., 1991; Wallingford et al., 2013). In these cases, changes in the geometry and mechanics of constituent cells appear to impact global tissue shape directly (Odell et al., 1981; Escudero et al., 2007; Sherrard et al., 2010; Eiraku et al., 2011). Apical constriction of individual cells can contribute to cell ingression from epithelial tissues, sometimes as a step in an epithelial-mesenchymal transition (EMT) (Anstrom, 1992; Nance and Priess, 2002; Harrell and Goldstein, 2011; Williams et al., 2012). Apical constriction is also associated with the extrusion of apoptotic or delaminating cells (Toyama et al., 2008; Slattum et al., 2009; Marinari et al., 2012) and wound contraction and healing (Davidson et al., 2002; Antunes et al., 2013). Thus, apical constriction remodels epithelia in a variety of ways to achieve proper tissue shape and structure.

Given the different contexts in which apical constriction functions, the question arises as to whether there is a universal force-generating mechanism that underlies apical constriction for all cell types. Signals that promote apical constriction appear to converge on a core set of cytoskeletal and adhesion proteins that generate and transmit the forces driving both apical constriction and other cell shape changes (Sawyer et al., 2010; Mason and Martin, 2011). Contraction of actin filament (F-actin) networks by the molecular motor non-muscle myosin II (referred to hereafter as myosin) has long been implicated in generating the force that drives apical constriction (Box 1; Fig. 2). To elicit apical constriction, contractile forces generated by the actin-myosin cytoskeleton are exerted on the apical circumference and between cells through attachments between the actin network and sites of cell-cell adhesion, such as adherens junctions (AJs; Box 2; Fig. 2).

Although a common machinery involving actin, myosin and AJs is involved in apical constriction in diverse cell types, recent live-imaging studies have highlighted the variety of ways in which cell types regulate actin-myosin dynamics and AJs during apical constriction. Apical actin-myosin networks exhibit a wide variety of behaviors, including actin-myosin flows, contractile pulsing, and the formation of actin-myosin fibers, suggesting that this molecular 'engine' can be organized in different ways to generate force (Martin, 2010). Some cell types can dynamically change the apparent strength of coupling between actin-myosin networks and AJs, suggesting the presence of a molecular 'clutch' at AJs that modulates the ability of the actin-myosin engine to elicit cell shape

Box 1. Actin-myosin contraction

Contractile force in cells predominantly results from collective interactions between type II myosin motors (referred to hereafter as myosin) and actin filaments (for reviews, see Lecuit et al., 2011; Salbreux et al., 2012). Myosin is a hexameric complex that consists of a pair of heavy chains, which contain the motor domains, and two pairs of light chains. Myosin molecules assemble tail-to-tail to form bipolar minifilaments with the motor domains at the ends of a central rod (Verkhovsky and Borisy, 1993). Myosin minifilament assembly and motor activity is regulated by phosphorylation of the myosin regulatory light chain. Multiple kinases, including Rho-associated coiled-coil kinase (ROCK), can phosphorylate myosin regulatory light chains and promote minifilament assembly, whereas myosin phosphatase dephosphorylates and inactivates the myosin motor (Sellers, 1991; Bresnick, 1999).

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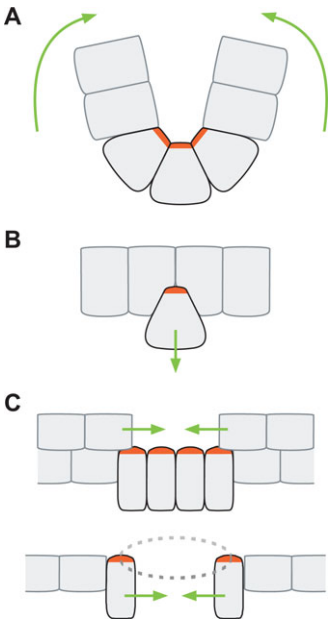

Fig. 1. Functions and examples of apical constriction. (A-C) Apical constriction functions in various contexts including: (A) tissue folding and tube formation, seen in examples of gastrulation and vertebrate neurulation; (B) ingression of individual cells and epithelial-to-mesenchymal (EMT) transitions, as occur in other examples of gastrulation and in tissue homeostasis; and (C) healing and sealing of embryonic tissues in response to wound healing. The cell and tissue movements (green arrows) that occur as specific cells undergo constriction of their apical sides (orange) are indicated in each context. Wound healing can involve apical constriction of an underlying layer of cells, or of a ring of cells (dashed line; just two such cells of the ring are drawn) at the periphery of a wound.

change (Roh-Johnson et al., 2012). Furthermore, some cell types undergo apical cell shape fluctuations, and the speed of apical constriction can vary dramatically, depending on the extent to which the apical domain relaxes after decreasing in area, suggesting a regulated cellular component that serves as a 'ratchet' to tune the dynamics of apical constriction (Martin et al., 2009; Solon et al., 2009; Blanchard et al., 2010). Thus, although the cellular machinery required for apical constriction appears to be the same for various cell types, the organization and dynamics of this cellular machinery can vary.

This Review focuses on how contraction and adhesion are tuned and coordinated to regulate apical constriction in diverse cellular contexts. We discuss the evidence supporting the importance of key dynamic components that have been inferred, such as pulses, a clutch and a ratchet, which may exist to differing extents in various cell types that undergo apical constriction. We hypothesize that these components define regulatory modules that cells may tune, adapting apical constriction to the different tissue remodeling events that rely on this common cell shape change.

The central force generators: contractile fibers, cortical flows and pulses during apical constriction

Actin-myosin contraction can generate cellular contractile force that is transmitted between cells. However, actin-myosin networks can exhibit differences in network architecture, such as linear bundles or two-dimensional networks. In addition, the components of actin-myosin networks can have varying dynamics, depending on the structure and cell type. Below, we discuss how actin-myosin networks with varying architectures and dynamics are utilized to promote apical constriction in different contexts.

Contractile fibers

Myosin generates contractile force by pulling actin filaments oriented in an antiparallel manner together. In the simplest form, contractile units composed of myosin and antiparallel F-actin bundles, called sarcomeres, are connected in series to generate contractile fibers, such as stress fibers (Tojkander et al., 2012). Stress fibers generate isometric tension that exerts traction forces on the external environment and maintains cell shape (Kumar et al., 2006). In addition, contractile units may be present in actin-myosin fibers without a clear sarcomeric repeat, such as in the cytokinetic ring (Carvalho et al., 2009). Classic studies of neurulation in embryos and of cultured epithelia suggested that the contraction of circumferential actin-myosin bundles or fibers underlying the adherens junctions causes the cell apex to constrict, analogous to drawing a purse-string (Baker and Schroeder, 1967; Burnside, 1973; Burgess, 1982; Owaribe and Masuda, 1982). A recent study demonstrated that circumferential actin-myosin bundles can exhibit a clear sarcomeric organization in several epithelial tissues *in vivo* (Ebrahim et al., 2013). Myosin activity in these tissues reduces the distance between sarcomeric repeats, consistent with myosin motor activity pulling antiparallel F-actin arrays together, driving a purse-string-like contraction. In adjacent cells, the repeated pattern of myosin and F-actin cross-linking proteins in F-actin bundles is aligned across the junctional interface, suggesting that individual units of this sarcomeric repeat are somehow coordinated and possibly anchored across junctional interfaces (Ebrahim et al., 2013). Cell culture studies have shown that actin-myosin fibers without a clear sarcomere organization can also generate tension around the apical perimeter and can drive apical constriction (Ishiuchi and Takeichi, 2011; Ratheesh et al., 2012; Wu et al., 2014). Thus, the contraction of linear actin-myosin fibers, such as those that circle the apical circumference, is a mechanism by which cells can generate force for apical constriction.

Cortical flows

Live imaging of apical constriction during development has suggested the additional importance of a more two-dimensionally organized actin-myosin network that underlies the plasma membrane, termed the actin cortex (Fig. 2) (Lecuit et al., 2011; Salbreux et al., 2012). The cortical actin-myosin network spans the apical surface of most epithelial cells, analogous to the terminal web of brush border epithelia. Although cortical actin-myosin networks lack the well-defined polarity and organization of some stress fibers, actin cortex contraction by myosin generates cortical tension, which can initiate cytoplasmic flows, compress the underlying plasma membrane and generate traction forces on external substrates (Aratyn-Schaus et al., 2011; Sedzinski et al., 2011; Kapustina et al., 2013). In addition, cortical contraction results in the lateral movement of components of the actin-myosin cytoskeleton and the associated plasma membrane proteins from regions of low tension to areas where the network generates higher tension; this is termed cortical flow (Bray and White, 1988; Mayer et al., 2010; Goehring and Grill, 2013). During cortical flow, individual components of the actin-myosin network can undergo turnover (Ponti et al., 2004). For example, F-actin depolymerization during contraction contributes to the pool of actin monomers, which re-polymerize, thereby maintaining a continuous F-actin network underlying the plasma membrane. The constant turnover of F-actin and myosin may allow these contractile networks to maintain gradients of active tension and flow, resulting in a contractile engine generating inward-directed forces that can be harnessed to elicit cell shape changes.

How cortical actin-myosin networks contract without having well-defined F-actin and myosin orientation and contractile units formed

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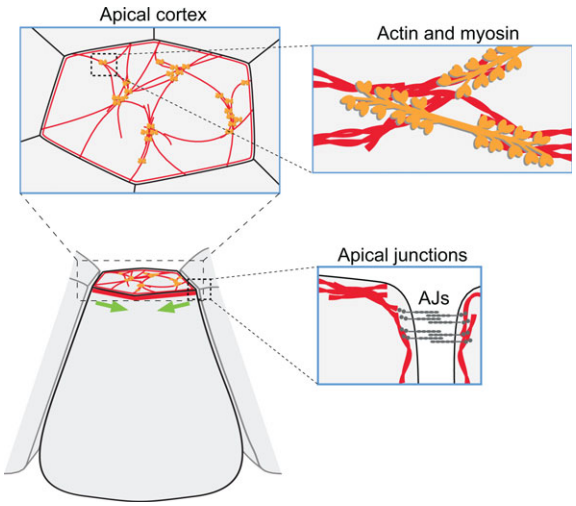

Fig. 2. Mechanisms of apical constriction. Key components involved in apical constriction include F-actin (red) and myosin (orange), which form contractile networks. Actin-myosin networks can be organized into contractile bundles/fibers or can be organized into a more loosely organized two-dimensional network that underlies the plasma membrane, called the apical cortex. Shrinkage of the apical cortex (green arrows) is driven by actin-myosin contractions. Apical adherens junctions (AJs, gray) link cells, allowing apical actin-myosin contractions to drive tissue shape changes. In this example, only the apical actin cortex is shown.

a priori is not well understood. Despite having initially random F-actin orientations, reconstituted actin-myosin networks contract on length scales that are typical of a cell apex (~10 µm) (Bendix et al., 2008; Soares e Silva et al., 2011; Murrell and Gardel, 2012; Carvalho et al., 2013). This suggests that an inherent actin filament polarity in the network is not a prerequisite for contraction. It has been proposed that contraction results from the asymmetric response of actin filaments to tensile and compressive stress: actin filaments in contracting networks can resist high levels of tension, and thus can be pulled, but easily buckle under compression, meaning that the network will preferentially shrink rather than expand (Soares e Silva et al., 2011; Murrell and Gardel, 2012). Consistent with this model, the extent of network contraction has been shown to correlate with individual actin filament buckling in a reconstituted system (Murrell and Gardel, 2012). In addition, actin-myosin networks can exhibit multistage coarsening behavior and the self-organization of foci that are concentrated in myosin and surrounded by F-actin and that coalesce to form higher order clusters of myosin and F-actin (Soares e Silva et al., 2011). *In vivo* cortical actin-myosin networks also have a coarse architecture with myosin foci and surrounding F-actin, suggesting that either regulated assembly or self-organization within the actin-myosin cortex could facilitate cortical flow and force generation (Munro et al., 2004, Vavylonis et al., 2008; Luo et al., 2013).

Actin-myosin network contraction and the associated cortical flow have been observed in several instances of apical constriction. For example, convergent movements of myosin and other apical proteins have been observed, where these proteins coalesce into larger foci (Martin et al., 2009; Roh-Johnson et al., 2012; David et al., 2013). This flow can be visualized during *Caenorhabditis elegans* gastrulation, in which multiple myosin foci are maintained over long periods of time, allowing fine mapping of cortical myosin movements. In *C. elegans*, gastrulation begins when two endodermal precursor cells internalize from the surface of the embryo at the 26- to 28-cell stage (Fig. 3A). These two cells internalize by apical constriction: myosin becomes activated in the apical cortex of these cells (Fig. 3B), and F-actin and myosin activity are required for cell internalization (Lee and Goldstein, 2003; Lee et al., 2006). During apical constriction, the apical actin-myosin network flows predominantly centripetally, towards the center of the apical cortex. Network components are added continuously, with new myosin foci forming disproportionately near the perimeter of the apical cortex, resulting in a conveyor belt-like centripetal flow (Roh-Johnson et al., 2012). Consistent with the relevant force generators lying throughout the apical actin-myosin network, points on the network near the edges of contractions in *C. elegans* have been seen to move centripetally faster than do points closer to the center, as is the case for any sheet of material with contractile elements scattered throughout (Munro et al., 2004; Roh-Johnson et al., 2012). When the apical domain shrinks, apical myosin movements occur in concert with the apical circumference, consistent with cortical actin-myosin flow serving as an engine that contracts cell apices.

Pulses

Many cases have now been documented in which the myosin flow and/or assembly of actin and myosin structures during apical constriction occur as discrete events, or pulses (Martin et al., 2009; Blanchard et al., 2010, David et al., 2010; Azevedo et al., 2011). Actin and myosin pulses are also observed in non-apical regions of cells and appear to play roles in a wide variety of cell and tissue shape changes (Munro et al., 2004; Skoglund et al., 2008; He et al., 2010, Kim and Davidson, 2011; Sedzinski et al., 2011; Kapustina et al., 2013). Several pieces of evidence suggest that myosin pulses represent transient contraction of the actin-myosin networks. First, actin and myosin become locally recruited and/or condensed in the apical cortex simultaneously (Blanchard et al., 2010; He et al., 2010; Mason et al., 2013). Second, pulses of actin and myosin enrichment are correlated with phases of cell constriction and appear to pull junctional structures inwards (Martin et al., 2009; Blanchard et al., 2010; He et al., 2010; Kim and Davidson, 2011). Third, disruption of actin-myosin contractility prevents associated actin reorganization and cell constriction (Martin et al., 2009; He et al., 2010; Kim and Davidson, 2011; Mason et al., 2013). Thus, myosin pulses could represent a cellular-scale power stroke of the actin-myosin engine, driving rapid, if transient, cell shape changes.

Box 2. Adherens junctions

Adherens junctions serve as points of cell-cell attachment that also anchor the actin cortex to the apical circumference of the cell, allowing contractile forces to be transmitted between cells (for a review, see Niessen et al., 2011). Adherens junctions in epithelial cells contain the homophilic cell adhesion molecule E-cadherin. The extracellular domain of E-cadherin mediates cell-cell adhesion, whereas its intracellular tail forms a complex with two other proteins, β-catenin and α-catenin. Because α-catenin binds actin filaments, the E-cadherin–β-catenin–α-catenin complex is thought to link adherens junctions to the actin cortex. Although biochemical studies suggest that mammalian α-catenin cannot bind β-catenin and F-actin at the same time (Drees et al., 2005; Yamada et al., 2005), it remains possible that this linkage is regulated in a way that biochemical experiments do not fully reconstitute. The E-cadherin complex might be linked to F-actin via additional proteins, such as EPLIN (also known as Lima1) (Abe and Takeichi, 2008), vinculin (Yonemura et al., 2010; le Duc et al., 2010), afadin (also known as Mllt4) (Pokutta et al., 2002; Sawyer et al., 2009; Toret et al., 2014), ZO-1 (also known as Tjp1) (Itoh et al., 1997), α-actinin (Knudsen et al., 1995) or β-spectrin (Pradhan et al., 2001). In addition, other adhesion protein complexes may function in parallel with classical cadherin complexes (Sawyer et al., 2009; Morita et al., 2010).

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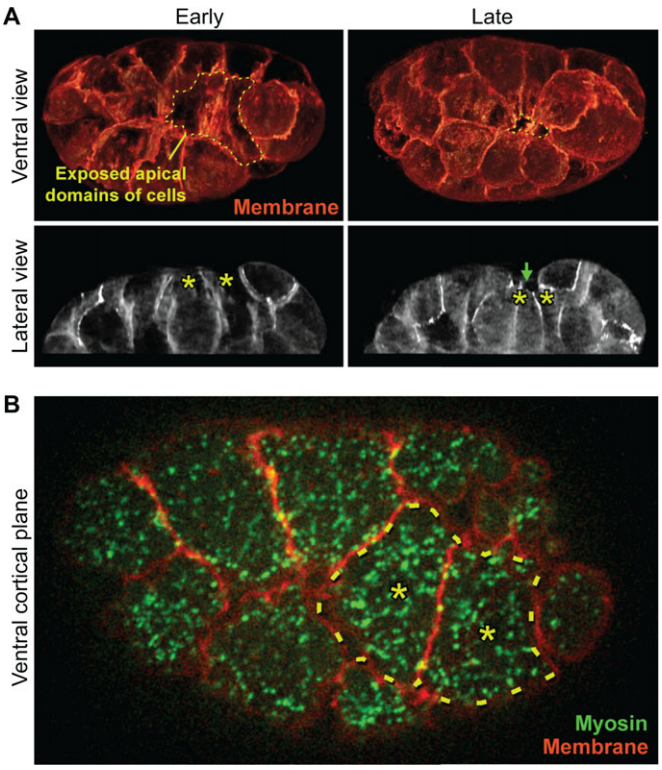

Fig. 3. Apical constriction by cortical actin-myosin flows. (A) Apical constriction in *C. elegans* gastrulation. Membranes, which are marked with an mCherry (red) membrane marker, were imaged using Bessel beam super-resolution structured illumination microscopy (Gao et al., 2012). Images courtesy of C. Higgins and L. Gao. Two stages (early and late) are shown to highlight the internalization of the two endodermal precursor cells, the exposed apical domains of which are indicated (yellow dashed lines) at each stage. Below, optical sections of the same embryos at the same stages are shown. Endodermal precursor cells are marked (asterisks), and the direction of their internalization is indicated (green arrow). (B) A *C. elegans* embryo (~50 µm long) in which the apical constriction of endoderm precursor cells has begun. The embryo expresses GFP-tagged myosin (green) and an mCherry-tagged plasma membrane marker (red). This spinning disk confocal image shows the large number of myosin foci visible in the apical cortex of endoderm precursor cells (asterisks; apical domain outlined by yellow dashed line). Image courtesy of C. Higgins.

What then causes actin-myosin contractions and associated flow to be pulsatile rather than continuous?

Oscillations in biological systems often result when the activation of a cellular process or signal also results in delayed negative feedback (Morgan, 2006). In systems with pulsatile apical constriction, feedback could result from the recruitment of a negative regulator of contractility, and/or by mechanical feedback. Evidence for both models exists. For example, during dorsal closure in *Drosophila*, cells of the amnioserosa (an epithelium that occupies the dorsal side of the embryo) undergo apical constriction during late embryogenesis and exhibit dramatic fluctuations in apical areas that are associated with actin-myosin pulses. Laser ablation of apical actin-myosin cortices of individual amnioserosa cells or the mosaic expression of proteins that inhibit contractility inhibit oscillations in neighboring amnioserosa cells (Solon et al., 2009; Saravanan et al., 2013). It has also been shown that, in the *Drosophila* egg chamber, the periodicity of basal actin-myosin pulses is affected by the strength of cytoskeleton-adhesion attachment, with increased attachment strength delaying the time between myosin pulses (He et al., 2010). Thus, data in these two systems suggest that mechanical forces, possibly tension transmitted across the cytoskeletal network, influences the contractile cycle via as yet undefined mechanisms. In addition to mechanical forces, apical domain oscillations in *Drosophila* amnioserosa cells are influenced by proteins that define apical-basal cell polarity. Inactivation of a key component of the apical Par complex, atypical protein kinase C (aPKC), results in a higher pulse frequency, whereas ectopic aPKC activation decreases pulse frequency (David et al., 2010). Thus, aPKC appears to inhibit myosin pulses, consistent with a role in inhibiting myosin contractility in other contexts (Ishiuchi and Takeichi, 2011; Röper, 2012). Interestingly, actin-myosin networks recruit apical aPKC, providing a possible negative feedback loop that could promote oscillations in myosin activity that drive pulsatile contractions (David et al., 2013). The different mechanisms that regulate pulsing, and the fact that actin-myosin contractions can be more or less pulsatile, illustrate how the actin-myosin engine that drives apical domain shrinkage can be uniquely tuned in different contexts.

The importance of location: differential localization of key signals that regulate apical constriction

Different cell types that undergo apical constriction assemble actin-myosin structures in distinct regions of the apical cortex. Many epithelial cells exhibit a belt of F-actin and myosin at the apical circumference underlying adherens junctions (referred to as 'junctional' or 'circumferential'; Fig. 4A) without having prominent myosin structures on the apical cortex. By contrast, other cell types preferentially accumulate actin-myosin in the middle of the apical domain (referred to as 'medial' or 'medioapical'; Fig. 4A) where actin-myosin structures span junctions present on opposite sides of the apical actin cortex. How are actin-myosin structures targeted to these distinct apical regions, and is the spatial control of contractility within the apical domain important for apical constriction? As discussed below, recent studies of apical constriction in various contexts have revealed how the spatial localization of upstream regulators of contractility dictates the nature of the applied contractile force.

Medioapically localized contractility

Cell contractility is often regulated via the small GTPase RhoA. In its active, GTP-bound form, RhoA binds to and activates numerous effectors that affect many cell processes, including the actin-myosin cytoskeleton (Jaffe and Hall, 2005). RhoA activates Rho-associated coiled-coil kinase (ROCK), which promotes myosin activation both by inhibiting myosin phosphatase and by directly phosphorylating myosin regulatory light chain (Bresnick, 1999). In addition, RhoA can activate Diaphanous (Dia)-related formins, which nucleate and facilitate the assembly of unbranched actin filaments (Goode and Eck, 2007). Apical constriction in several vertebrate and invertebrate systems requires RhoA GTPase activation (Barrett et al., 1997; Hacker and Perrimon, 1998; Plageman et al., 2011; Nishimura et al., 2012). In *Drosophila* gastrulation, RhoA (Rho1 in *Drosophila*) activation plays a central role in a signaling pathway that links tissue patterning and cell fate to apical constriction and cell invagination (Leptin, 2005). Apical constriction of the presumptive mesoderm cells promotes epithelial bending, promoting the formation of a ventral furrow and the subsequent invagination of these ventral cells (Sweeton et al., 1991; Kam et al., 1991). The identified signaling pathway is initiated by the transcription factors Snail and Twist, which cooperatively activate G protein-coupled receptor signaling and recruit the PDZ-RhoGEF guanine nucleotide exchange factor (DRhoGEF2 in *Drosophila*) to the apical surface where it activates RhoA (Fig. 4B) (Costa et al.,

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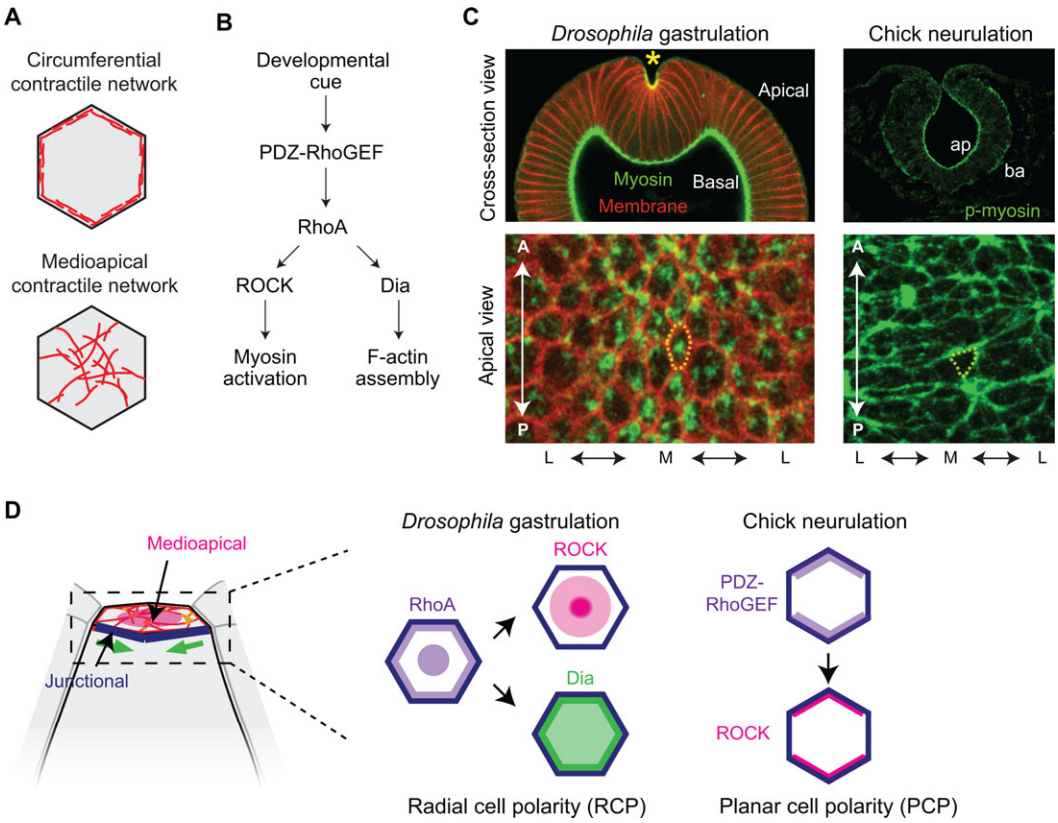

Fig. 4. Variations in the spatial localization of actin-myosin structures during apical constriction. (A) Schematic illustrating the location of circumferential actin-myosin networks and medioapical actin-myosin networks (both illustrated in red). (B) A conserved pathway, involving PDZ-RhoGEF, RhoA, ROCK and Diaphanous (Dia), regulates myosin activation and F-actin assembly in *Drosophila* mesoderm cells and the chicken neural tube. (C) Cross-sections (top) and apical surface (bottom) views of *Drosophila* mesoderm cells during gastrulation (left) and of the chick neural tube (right). Myosin (green) is preferentially in the medioapical domain in the *Drosophila* presumptive mesoderm cells, but is preferentially at junctions during chick neurulation. Asterisk marks the site of invagination, where mesoderm precursor cells are undergoing apical constriction. In the apical views, a single cell is outlined (yellow dotted line). Axes are also marked: apical-basal (ap-ba), medial-lateral (M-L) and anterior-posterior (A-P). *Drosophila* cross-section courtesy of C. Vasquez and neural tube images courtesy of M. Takeichi (Nishimura et al., 2012). (D) Key components of the RhoA pathway exhibit different spatial organizations during *Drosophila* gastrulation and vertebrate neurulation. During *Drosophila* gastrulation, mesoderm cells exhibit a radial cell polarity (RCP) in which RhoA (purple) and its effector ROCK (pink) are present in a medioapical focus. RhoA is also present at junctions. Dia (green) is present at junctions and throughout the apical cortex. During chick neurulation, PDZ-RhoGEF (purple) and ROCK (pink) are localized at junctions and exhibit planar cell polarity (PCP). In left-hand diagram, green arrows indicate constriction.

1994; Parks and Wieschaus, 1991; Kolsch et al., 2007; Manning et al., 2013). ROCK (Rok in *Drosophila*) and the formin Dia are required for efficient apical constriction (Dawes-Hoang et al., 2005; Homem and Peifer, 2008), consistent with both myosin activation and F-actin assembly being coordinately regulated downstream of RhoA to generate contractile force. Thus, the *Drosophila* mesoderm is a well-characterized example of apical activation of RhoA promoting apical constriction.

Rather than forming a circumferential actin-myosin belt, *Drosophila* presumptive mesoderm cells preferentially accumulate myosin in the medioapical cortex (Young et al., 1991; Dawes-Hoang et al., 2005; Martin et al., 2009) (Fig. 4C). Imaging-based analysis of components of the RhoA pathway demonstrated that RhoA, Dia and ROCK have distinct spatial distributions in the apical domain (Fig. 4D) (Mason et al., 2013). RhoA and ROCK aggregates or foci are often concentrated near the center of the medioapical cortex. In addition, Dia localizes across the apical cortex, often overlapping with ROCK foci. Although RhoA and Dia are also present at cell-cell junctions around the apical circumference, ROCK appears to be excluded from the AJ domain. Thus, *Drosophila* mesoderm cells exhibit a type of polarity, termed radial cell polarity (RCP), in which ROCK/myosin and junctional proteins are enriched in complementary regions of the apical surface. Evidence suggests that the localization of ROCK and AJ proteins within the apical domain is important for apical constriction. First, *dia* mutants cause E-cadherin (Shotgun in *Drosophila*) to become localized across the entire apical domain rather than being restricted to the apical circumference (Mason et al., 2013). This depolarization of E-cadherin is associated with transient uncoupling of contractile networks of neighboring cells, suggesting an intriguing possibility that restricting adhesion proteins to the circumference is important to anchor the actin-myosin cortex to the apical margin. Second, *twist* and *snail* mutants result in an inversion of ROCK polarity, with ROCK and myosin becoming mislocalized to junctions rather than to medioapical foci (Mason et al., 2013). The control of the location of contractile signals within the apical domain by the transcriptional targets of Twist and Snail suggests that the positioning of contractile elements and the resulting force generation are crucial for organizing the constriction of the cell apex. The mechanism that establishes RCP is still unknown. One possibility is that RhoA activity is spatially and temporally coordinated with other Rho-family GTPases, as is seen in the case of cell migration (Machacek et al., 2009). Mutual antagonism between the RhoA and Rac1 GTPases has been observed during apical constriction in the vertebrate lens placode (Chauhan et al., 2011), although the spatial organization of active

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RhoA and Rac1 is not known for either the vertebrate or invertebrate systems. Thus, it will be interesting to determine how signals, such as RhoA and Rac1, are spatially and temporally coordinated to control actin-myosin contractility in different cell types and to understand the consequences of this spatial control on apical constriction.

Circumferential actin-myosin belts

In contrast to the situation observed during *Drosophila* gastrulation, apical constriction during vertebrate neural tube formation is associated with the contraction of the actin-myosin networks that underlie junctions (Baker and Schroeder, 1967; Burnside, 1973). Neural tube formation involves apical constriction of cells in defined locations of the neural epithelium, called hingepoints, resulting in the folding of the neural plate (Copp and Greene, 2010). A gene originally called *shroom* (now referred to as *Shroom3*) is required for apical constriction and the proper bending of the neural epithelium in various vertebrate embryos (Hildebrand and Soriano, 1999; Haigo et al., 2003; Lee et al., 2007; Nishimura and Takeichi, 2008). In addition, transcription of Shroom3 has been shown to result in apical constriction and epithelial morphogenesis in several other vertebrate tissues (Plageman et al., 2010; Chung et al., 2010; Ernst et al., 2012). Shroom3 is an F-actin binding protein that localizes to apical junctions (Hildebrand and Soriano, 1999; Hildebrand, 2005; Nishimura and Takeichi, 2008). Shroom3 binds and recruits ROCK to junctions, resulting in the formation of actin-myosin cables that sometimes exhibit a sarcomere-like repeat pattern around the apical domain (Hildebrand, 2005; Nishimura and Takeichi, 2008). Shroom3-mediated myosin assembly at junctions appears to increase circumferential tension, as evidenced by the straightening of cell-cell interfaces and the apical constriction of individual cells in a sheet of cells expressing Shroom3 (Hildebrand, 2005). By contrast, Shroom3 truncations that lack the F-actin binding domain and that are ectopically targeted throughout the apical surface of Madin-Darby canine kidney (MDCK) cells result in the formation of radial myosin fibers that appear to pull junctions inwards (Hildebrand, 2005). Furthermore, *Drosophila* Shroom is present as long and short isoforms that exhibit circumferential and medioapical localization, respectively (Bolinger et al., 2010). The localization of these Shroom isoforms correlates with the position of myosin localization when ectopically expressed. Thus, spatial regulation of myosin by Shroom proteins appears to provide a control point to direct contractility to either junctions or the medioapical cortex.

Although Shroom3 can target myosin to junctions, studies of neural tube formation in chicken embryos have demonstrated that myosin, but not Shroom3, is enriched in a subpopulation of junctional interfaces (Nishimura and Takeichi, 2008). Activated myosin is enriched at junctions that are aligned with the medial-lateral axis of the embryo (Fig. 4C), and thus exhibits planar cell polarity (PCP) (Nishimura et al., 2012). The PCP of actin-myosin cables in the neural epithelium could polarize tissue contraction in the medial-lateral direction such that the tissue can fold while also extending in the anterior-posterior axis. The vertebrate PDZ-RhoGEF (also known as Arhgef11), which is required for proper neural tube folding, exhibits planar cell polarized enrichment in medial-lateral junctions (Nishimura et al., 2012). Thus, whereas the RhoA pathway and myosin exhibits RCP during *Drosophila* gastrulation, PDZ-RhoGEF, ROCK and myosin exhibit PCP during vertebrate neural tube formation (Fig. 4D). Because Shroom3 is important for neural tube formation (Hildebrand and Soriano, 1999; Haigo et al., 2003; Nishimura and Takeichi, 2008), the question remains of how RhoA and Shroom3 cooperate to control ROCK localization and/or activity. RhoA and Shroom3 bind distinct regions of ROCK, presenting the possibility that ROCK can integrate these two signals to spatially control contractility (Nishimura and Takeichi, 2008; Plageman et al., 2011). In the eye lens placode, RhoA is necessary for Shroom3 localization and basolateral RhoA activation can ectopically recruit Shroom3 (Plageman et al., 2011). However, dominant-negative RhoA does not block Shroom3-mediated apical constriction in other cell types (Haigo et al., 2003; Hildebrand, 2005), suggesting that Shroom3 can mediate Rho-independent ROCK activation in other contexts. Overall, these studies highlight how conserved signals, such as RhoA, Shroom3 and ROCK, are differentially localized to induce apical constriction via distinct actin-myosin arrangements.

Linking to the apical circumference: evidence for a dynamically regulated connection between apical actin-myosin and intercellular junctions

For actin-myosin contraction to result in a cell shape change, apical actin networks must be coupled to AJs at the apical circumference. Experiments in which AJ components are disrupted have resulted in actin-myosin contractions that fail to pull the apical circumference inwards efficiently (Dawes-Hoang et al., 2005; Sawyer et al., 2009; Roh-Johnson et al., 2012). During *Drosophila* gastrulation, disruption of AJ components results in a medioapically localized bolus of actin and myosin, and depletion of β-catenin or α-catenin has been shown to result in continuous myosin flow into this bolus, with myosin flow being uncoupled from movement of the plasma membrane at apical cell-cell contacts (Dawes-Hoang et al., 2005; Sawyer et al., 2009; Martin et al., 2010). Similar experiments in *C. elegans* do not result in a concentrated medioapical bolus of actin and myosin, but instead give rise to centripetal myosin flow that fails to move in concert with the apical membrane contacting adjacent cells (Roh-Johnson et al., 2012). These experiments confirm a simple expectation: junctional components are necessary to efficiently link apical cell-cell contacts to contracting actin-myosin networks, and hence to accomplish cell shape change.

More surprising was the finding that links between apical cell-cell junctions and contracting actin-myosin networks are not constitutive, but instead appear to be temporally regulated (Roh-Johnson et al., 2012). In gastrulating embryos of both *C. elegans* and *Drosophila*, actin-myosin contractions go on for minutes before the apical sides of cells begin to shrink. In *C. elegans* endoderm precursor cells, the overlying plasma membrane has been found to move along with the conveyor belt-like centripetal movement of actin-myosin. Populations of myosin particles are continuously added near the edge of the apical cortex and move generally towards the center of the apical surface for several minutes before junctional membranes begin to move in concert with myosin particles (Fig. 5) (Roh-Johnson et al., 2012). Rates of myosin movements are greater in cells undergoing apical constriction than in other cells, consistent with greater myosin activation in the constricting cells (Lee et al., 2006). However, rates of myosin movements do not increase as apical constriction begins, suggesting that an upregulation of myosin activity is not the trigger for initiating apical constriction.

If an increase in myosin activity does not appear to trigger apical constriction, is there an increase in apical tension that is produced by other means? Laser-cutting experiments, which can be used to estimate both tension and stiffness in the apical network, demonstrated that high levels of tension are present in the apical cortex of endoderm precursors prior to constriction and that neither tension nor stiffness changes as the cell apices begin to shrink (Roh-Johnson et al., 2012). This result implies that apical constriction of

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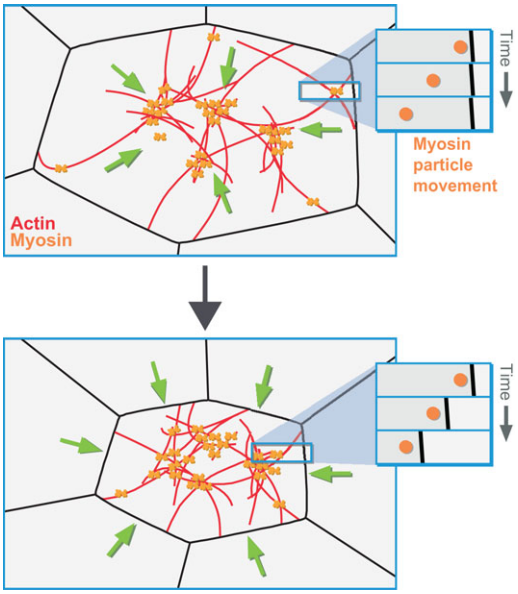

Fig. 5. Evidence for dynamic regulation of connections between the actin-myosin cortex and apical junctions. At the onset of gastrulation in *C. elegans* and *Drosophila* (top), myosin (orange) and the associated network initially flows centripetally without moving apical junctions in concert and, hence, without causing shrinking of the apical domain. Centripetal actin-myosin contraction is represented by green arrows. Later (bottom), cortical actin-myosin flow moves more in concert with apical junctions (green arrows), which converge, shrinking the apical surface.

C. elegans endoderm cells is not triggered by an increase in apical tension. The finding that tension does not change as apical constriction begins also implies that the tension present in the actin-myosin network, even prior to gastrulation, is large compared with the amount of force required to pull neighboring cells. This early tension might result from stresses present between the actin-myosin network and cellular components to which the network is linked, for example by protein links to the overlying membrane, friction with the underlying cytoplasm, and/or direct, continuous links to the basolateral cortical actin-myosin network. The strong forces present in this system compared with the small difference in forces apparently required for productive movement may be a general theme of biological systems on this scale. Strong, counteracting forces have been demonstrated by laser cuts in *Drosophila* embryos during dorsal closure, and in mitotic spindles of dividing cells before anaphase, also cases in which movement is accompanied by an apparently small imbalance among comparatively strong forces (Aist and Berns, 1981; Kiehart et al., 2000; Grill et al., 2001; Hutson et al., 2003). Taken together, these results imply that neither the activation of myosin-based motility nor an associated increase in apical tension can be the proximate trigger for the apical constriction of *C. elegans* endoderm cells.

What then is the proximate trigger for apical constriction to begin? One model is that neighboring cells might lose apical tension, allowing the tension in the apical cortex of endodermal precursor cells to result in constriction. However, laser cuts have shown that the low level of tension in neighboring cells does not decrease further as apical constriction begins (Roh-Johnson et al., 2012). Alternatively, cell division in neighboring cells might relieve tension on cells undergoing apical constriction, because dividing cells have been shown to spread along the surface of the embryo (Pohl et al., 2012; Chihara and Nance, 2012). However, divisions of neighboring cells are unlikely to contribute significantly to apical constriction of the endoderm precursor cells in *C. elegans*, because most of the apical cell shape changes occur when neighboring cells are not dividing (Roh-Johnson et al., 2012). Given these results, and the finding that apical actin-myosin networks contract, moving overlying membranes but specifically failing at first to pull membranes at apical junctions inwards, it is likely that links between the apical actin-myosin network and the membranes at apical cell-cell junctions are temporally regulated, acting as a clutch that engages membrane movement with actin-myosin contractions. These findings have refocused efforts to understand how the dynamic regulation of key actin-membrane links might act to trigger apical constriction.

Maintaining shape: mechanisms that stabilize the cell shape fluctuations generated by contractile events

For actin-myosin contraction to elicit apical constriction, the reduced size of the apical domain must be stabilized against internal or external stresses to prevent relaxation back to the original shape. This is especially true during pulsatile contractions during which actin-myosin networks are disassembled or otherwise remodeled between pulses. Cells must be able to stabilize apical shape between contractile pulses such that repeated pulses will result in net constriction of the apical domain. This principle is illustrated by dorsal closure of the *Drosophila* epidermis during embryogenesis (Fig. 6A). Dorsal closure is influenced by forces generated by two different embryonic tissues: the dorsally positioned amnioserosa and the lateral epidermis (Kiehart et al., 2000; Hutson et al., 2003). Cells at the leading edge of the dorsally migrating epidermis assemble an actin-myosin cable at the interface between these two tissues, exhibiting tension perpendicular to the direction of movement (Fig. 6B) (Young et al., 1991; Kiehart et al., 2000; Hutson et al., 2003). Thus, contractile activity of the actin-myosin cable of the epidermis could function like a supracellular purse-string that helps pull the epidermis dorsally (Edwards et al., 1997; Franke et al., 2005). In addition, extra-

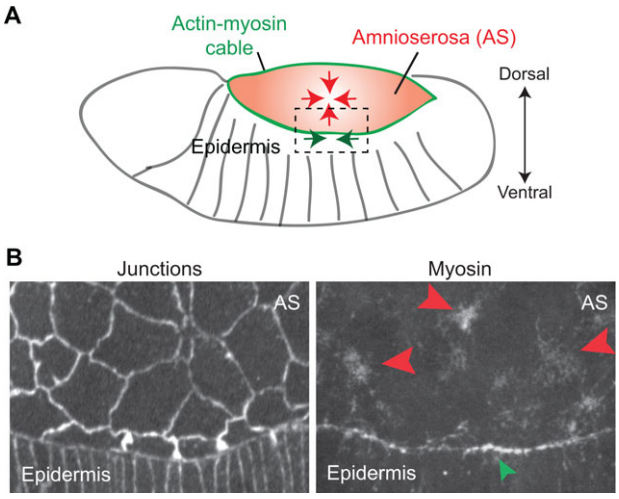

Fig. 6. Organization of contractile tissues during *Drosophila* dorsal closure. (A) Closure of the dorsal 'hole' in the *Drosophila* epidermis is driven by contraction of amnioserosa (AS) cells that occupy this hole (red arrows) and by contraction of a supracellular actin-myosin cable present at the leading edge of the epidermis (green arrows). (B) Morphology of the apical domain and of apical myosin in AS cells and the epidermis. AS cells have large apical domains that exhibit pulsed accumulations of myosin (red arrowheads, myosin accumulation in different AS cells). By contrast, epidermis cells exhibit myosin accumulation at the junctional interface with the AS cells (green arrowhead). Images represent different embryos. Images courtesy of T. Harris.

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embryonic amnioserosa cells occupy the dorsal hole in the epidermis and apically constrict, generating contractile forces that pull the epidermis dorsally (Kiehart et al., 2000). Amnioserosa cells exhibit dramatic pulsatile activity, undergoing cycles of cortical actin-myosin assembly and disassembly together with apical domain contraction and expansion (Fig. 6B) (Fernández et al., 2007; Solon et al., 2009; David et al., 2010; Blanchard et al., 2010; Azevedo et al., 2011; Sokolow et al., 2012). Pulsatile contractions and cell shape fluctuations begin 45 min to 1 h before dorsal closure, after which a developmental transition results in a more persistent contraction of the tissue. Why do amnioserosa cells initially expand after pulsatile contractions? One possibility is that amnioserosa cell expansion represents elastic strain in response to tension exerted by the surrounding tissue. Indeed, laser ablation studies have shown that dorsal closure results in tension in both the amnioserosa and the epidermis (Hutson et al., 2003; Solon et al., 2009). However, mechanically isolating cells by laser ablation of neighbors at different phases of the contractile cycle results in the isolated cells pausing or even continuing to expand, suggesting that amnioserosa cells are subjected to small elastic strains and that cell-intrinsic properties also contribute to expansion (Jayasinghe et al., 2013). Thus, amnioserosa cells probably need a mechanism to counteract both intrinsic and extrinsic stresses to prevent apical expansion following a contractile pulse.

How then do amnioserosa cells initiate apical constriction and dorsal closure? One model is that the actin-myosin cable that surrounds the amnioserosa tissue functions as a ratchet to counteract opposing forces that would expand the apical domain after a pulse (Solon et al., 2009). This model stems from the observation that, although amnioserosa cells appear to contract to a similar extent before and after dorsal closure initiates, amnioserosa cells near the actin-myosin cable progressively reduce the size to which they expand as the actin-myosin cable assembles and dorsal closure initiates. Supporting the role of the actin-myosin purse-string as a ratchet, a mutant in which actin-myosin cable formation is disrupted exhibits continual pulsing without a progressive reduction in apical domain size (Solon et al., 2009). The extrinsic effect of the actin-myosin cable could explain the spatial pattern of apical constriction, in which persistent constriction initially occurs at the periphery of the amnioserosa tissue and gradually moves inwards (Solon et al., 2009; Sokolow et al., 2012). However, whether the actin-myosin cable can serve as a ratchet to prevent global expansion of the entire amnioserosa tissue is still not clear.

Recent evidence suggests that changes in the intrinsic properties of amnioserosa cells can also promote apical constriction. Prior to dorsal closure, actin-myosin networks undergo cell shape fluctuations with large amplitudes and a periodicity of about 4 min (Blanchard et al., 2010; Sokolow et al., 2012). During dorsal closure, cell shape fluctuations become more frequent with a smaller amplitude. This transition is associated with increased apical myosin levels and a transition from cycles of actin-myosin network assembly and disassembly to more persistent apical actin-myosin networks (Fig. 7) (Blanchard et al., 2010; David et al., 2010, 2013). The alteration in apical actin-myosin dynamics could result from changes in the localization of apical polarity proteins, which become increasingly localized to the medioapical cortex over the course of dorsal closure (David et al., 2013). Thus, the extrinsic influence of the actin-myosin cable and intrinsic changes in actin-myosin dynamics of amnioserosa cells could cooperate to promote net apical constriction and collective tissue contraction.

In contrast to dorsal closure, apical constriction during *Drosophila* gastrulation occurs within minutes of apical myosin appearance. In

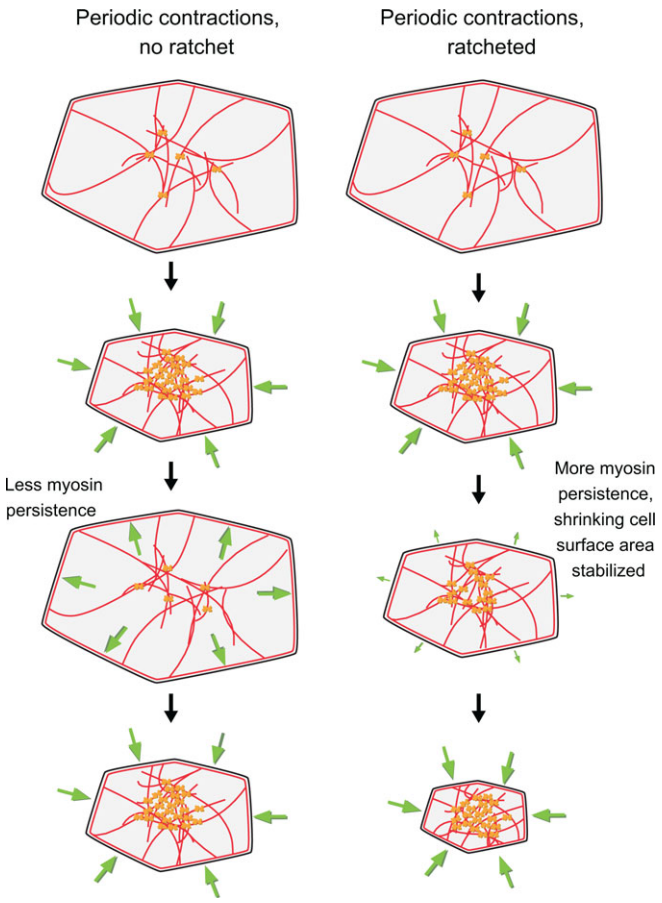

Fig. 7. Evidence for actin-myosin-dependent ratcheting of pulsed contractions. Pulsed contractions can occur without stabilization of the constricted apical surface area between pulses, resulting in cell shape fluctuations (left). These unratcheted constrictions have been observed in *Drosophila* amnioserosa cells before the onset of dorsal closure and in *Drosophila* presumptive mesoderm cells mutant for *twist*. Alternatively, decreases in apical surface area that result from contractile pulses can be stabilized, resulting in what sometimes resembles an incremental or ratchet-like decrease in apical area (right). This behavior is observed for wild-type presumptive mesoderm cells during *Drosophila* gastrulation and in amnioserosa cells during dorsal closure. In both these cases, both myosin and F-actin persist to a greater degree between pulses (right), which possibly prevents relaxation, resulting in a net reduction in apical surface area. Green arrows indicate shrinkage or expansion of apical domains.

Drosophila mesoderm cells, cell shape is maintained between contractile pulses such that cells constrict in a step-wise manner, much like a ratchet (Martin et al., 2009). Insight into the mechanism of this ratchet-like constriction has come from the fact that high levels of the transcription factor Twist are required to stabilize apical cell shape, but not for pulsatile contractions. As described above, Twist is required to polarize ROCK to medioapical foci (Mason et al., 2013). Medioapical ROCK and RhoA could potentially recruit and/or stabilize myosin and F-actin such that actin-myosin fibers can form across the apical domain. Consistent with this model, reducing Twist levels causes medioapical myosin and F-actin levels to decrease between pulses, rather than persistently accumulate (Martin et al., 2010; Mason et al., 2013). A consequence of this actin-myosin destabilization between pulses in *twist* mutants is the failure to accumulate a continuous supracellular meshwork of medioapical actin-myosin fibers that extends across the mesoderm tissue. Thus, Twist is required for persistent myosin and F-actin levels during apical constriction (Fig. 7), which appears to generate epithelial

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tension and stabilize cell shape between contraction pulses. The diverse mechanisms observed for *Drosophila* dorsal closure and gastrulation demonstrate that cell dynamics can be timed by modulating intrinsic and possibly extrinsic forces that stabilize cell shape fluctuations.

Possible roles for mechanical feedback and external constraints

Given that forces are transmitted between cells during apical constriction, mechanical signals have the potential to coordinate cell shape changes in a tissue by modulating the dynamic activities we have discussed, such as cortical flow, pulsing, contractile fiber formation, the arrangement of contractile structures, and the extent of coupling between the cortex and AJs. Several studies have suggested roles for mechanical feedback in regulating apical constriction. During *Drosophila* gastrulation, indentation of the embryo has been shown to rescue apical myosin accumulation in mutants that otherwise exhibit disrupted myosin activation (Pouille et al., 2009). The mechanical induction of myosin assembly could occur through regulation of the G protein-coupled receptor pathway that activates RhoA, with mechanical stimulation increasing plasma membrane tension and decreasing endocytic downregulation of the receptor, thus elevating pathway activity (Pouille et al., 2009). Recent studies of wound healing demonstrated that apical constriction in response to wounding is associated with cytoplasmic calcium accumulation through a stretch-activated calcium channel (Antunes et al., 2013). During *Drosophila* dorsal closure, calcium signaling has been shown to promote contractility, and two ion channels were identified as being required for embryos to regulate force generation after laser ablation (Hunter et al., 2014). In addition, mechanical signaling in *Drosophila* embryos induces the expression of the transcription factor Twist via accumulation of nuclear β-catenin (Farge, 2003; Desprat et al., 2008; Brunet et al., 2013), although it is not clear whether this transcriptional route of feedback could occur quickly enough to impact morphogenesis or rather impacts later cell fate decisions. Thus, an exciting area of future research will be to determine whether and how mechanical feedback regulates the dynamic activities that promote apical constriction discussed in this Review. Importantly, tension has been shown to impact both myosin contractility and junction strength in several other examples of cell and tissue morphogenesis (Ren et al., 2009; Fernandez-Gonzalez et al., 2009; Le Duc et al., 2010; Yonemura et al., 2010; Huveneers et al., 2012), suggesting that mechanical feedback plays an important role in coordinating cell behaviors during tissue morphogenesis.

In addition to modulating chemical signaling pathways, mechanical forces can impact the output of the contractile machinery by providing mechanical constraints on apical constriction. A classic example of this occurs during *Xenopus* gastrulation, during which cells at the site of blastopore formation undergo apical constriction and become shaped like bottles (Lee, 2012). Bottle cells are not conical, but rather are wedge-shaped, forming a groove for the invaginating blastopore. Importantly, the wedge shape depends on the presence of the adjacent tissue, suggesting that mechanical constraints of the tissue influence the final cell and tissue geometry (Hardin and Keller, 1988). A similar bias in the directionality of apical constriction was also shown for the *Drosophila* mesoderm, in which higher tension along the long axis of the furrow restricts apical constriction along this direction (Martin et al., 2010). A different type of constraint may be at work in the vertebrate eye, where two epithelia – the neural retina and the overlying lens placode – invaginate in close apposition to form a cup. In this case, thin actin-myosin-containing cellular protrusions connect the two epithelia during invagination, and disruption of these protrusions results in a reduction in the depth of lens cell invagination (Chauhan et al., 2009). In this case, the out-of-plane bending of one tissue could be mechanically coupled to that of another. Thus, it will be interesting in the future to determine how morphogenetic behaviors in different tissues are coupled and whether different mechanisms for apical constriction are associated with specific morphogenetic contexts.

Conclusions

Apical constriction functions in diverse physiological contexts to change tissue morphology. We hypothesize that cells in different organisms and tissues have, to some extent, tuned the activity of the processes that drive apical constriction in ways that fit specific morphogenetic processes. Evidence suggests that the positioning of actin-myosin networks and fibers (circumferential versus medioapical), their dynamics (pulsing versus continuous), the extent of coupling to AJs (weak versus strong) and the maintenance of apical cell shape between contractile events (relax versus ratchet) are all regulatable modules that might be combined to bring about different forms of apical constriction. It is still not clear why certain combinations of these regulatory modules might be present in one morphogenetic context versus another. Do different mechanisms such as pulsing versus continuous cytoskeletal contraction change the mechanics of the tissue or provide different ways of coordinating cell behavior? Does three-dimensional cell shape or the topology and mechanics of the surrounding tissue constrain different mechanisms for apical constriction? Or could these mechanisms give rise to distinct types of apical constriction that can be well coordinated with other cellular events that occur during development, such as epithelial rearrangements (e.g. intercalation or EMT), cell division, or differentiation? Greater knowledge of the mechanisms that underlie specific regulated modules may provide means to alter the dynamics of different apical constriction events and to test the importance of the various modes of force generation to morphogenesis.

Although our Review is focused on the roles of actin-myosin contractility and coupling to junctions, other cell processes undoubtedly need to be coordinated with cell contractility and adhesion to constrict cells effectively. Apical constriction by its definition is linked to apical-basal polarity, and changes in the activity of proteins that regulate apical-basal polarity can result in apical constriction, sometimes without an obvious increase in myosin activity (Rohrschneider and Nance, 2009; Fanning et al., 2012; Wang et al., 2012). Given the reduction of apical surface area, the removal of excess apical membrane could also play a crucial role in constriction (Lee and Harland, 2010; Mateus et al., 2011). In addition, the microtubule cytoskeleton is likely to play important roles in regulating cell morphology during apical constriction, having been implicated in apical-basal lengthening downstream of Shroom3, regulation of actin-myosin contractility through interactions with PDZ-RhoGEF, and control of cell-cell adhesion (Rogers et al., 2004; Lee et al., 2007; Lee and Harland, 2007; Suzuki et al., 2010). Finally, how cytoplasm, the nucleus and other organelles are influenced by apical constriction and vice versa is not well understood (Gelbart et al., 2012; Jayasinghe et al., 2013). Advances in microscopy and the increasing availability of molecular and biophysical tools to perturb gene/protein function and probe the mechanics of developing organisms means that we are likely to see progress and new surprises in years to come.

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