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Dynamics and regulation of contractile actin-myosin networks in morphogenesis

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Abstract

Contractile actin-myosin networks generate forces that drive cell shape changes and tissue remodeling during development. These forces can also actively regulate cell signaling and behavior. Novel features of actin-myosin network dynamics, such as pulsed contractile behaviors and the regulation of myosin localization by tension, have been uncovered in recent studies of *Drosophila*. *In vitro* studies of single molecules and reconstituted protein networks reveal intrinsic properties of motor proteins and actin-myosin networks, while *in vivo* studies have provided insight into the regulation of their dynamics and organization. Analysis of the complex behaviors of actin-myosin networks will be crucial for understanding force generation in actively remodeling cells and the coordination of cell shape and movement at the tissue level.

Introduction

Cell movements and cell shape changes are responsible for massive transformations in tissue structure during development. The actin-myosin cytoskeleton plays a major role in generating the forces that drive these changes and determining the mechanical properties of cells and tissues [1-3]. The non-muscle myosin II motor protein is a hexamer of three subunits (two heavy chains, two regulatory light chains, and two essential light chains) that converts the energy from ATP hydrolysis into mechanical work [4,5]. When actin and myosin form an interconnected network at the cell cortex, processive assemblies of myosin motors pull on anti-parallel actin filaments to generate contractile tension that can deform cell shape (Figure 1) [6-8]. In epithelial cells, contractile actin-myosin networks are coupled to adherens junctions, which mediate the transmission of forces between neighboring cells and integrate single cell behaviors to produce tissue-level changes during morphogenesis [9,10].

In addition to creating the forces that shape the embryo, the actin-myosin cytoskeleton is also a source of mechanical cues that regulate cell behavior, from cell differentiation and growth to cell shape and adhesion [11-15]. These forces are transduced into biochemical signals that influence cell behavior and modulate gene expression [12,13] and directly regulate the activity of motor proteins and the cytoskeleton [16-19].

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In comparison to typical biochemical signals that travel by diffusion or are transported by vesicles or molecular motors, mechanical perturbations propagate as sound waves whose speed depends on the mechanical properties and density of the material. Mechanical signals therefore have the potential to propagate rapidly over large distances. In one example, mechanical stress applied to human smooth muscle cells in culture activates the Src tyrosine kinase within a few hundred milliseconds, while Src activation by a soluble growth factor requires more than 10 seconds [20], suggesting the possibility that mechanical stimuli can activate signaling pathways faster than chemical stimuli. Thus, mechanical signals may be ideal for coordinating cell behaviors over the length and time scales relevant to the developing embryo.

Here we describe recent advances in understanding the dynamics of contractile actin-myosin networks that drive cell shape changes and tissue remodeling during development. We focus on recent studies in *Drosophila*, which have uncovered novel aspects of actin-myosin dynamics and regulation within the context of epithelial tissues *in vivo*. We discuss how the organization and dynamics of the contractile machinery lead to distinct properties of tissue structure as well as the mechanisms by which mechanical forces in turn regulate cell signaling and cytoskeletal activity. We also address how *in vitro* studies of the intrinsic dynamics and force-generating properties of actin-myosin networks can provide insight into the *in vivo* outcome of contractile behavior.

Organization and dynamics of the actin-myosin contractile machinery in multicellular tissues

The localization of the actin-myosin contractile machinery within cells is a key factor in determining the outcome of contractile activity for cell and tissue structure. One prominent example is the apical constriction of invaginating cells [7,21]. Initiated by the transcription factor Snail [22], prospective mesoderm cells on the ventral surface of the *Drosophila* embryo constrict their apical surfaces (Figure 2a,c), which generates a bend in the tissue that causes the cells to invaginate to form a ventral furrow at gastrulation (Figure 2a) [23,24]. These cell shape changes are associated with an actin-myosin network that spans the apical cell surface (Figure 2c), which we refer to as a medial myosin network. The medial myosin network in apically constricting cells is connected through a second, junctional population that is anchored to adherens junctions at cell-cell contacts (Figure 2c) [9,10,25,26]. Without this coupling to junctions, the medial network can contract into a tight ball without decreasing the apical surface, suggesting that this connection is essential to translate contraction of the medial network into a change in cell shape [24]. Although the intrinsic contractile activity of the apical network is isotropic, global tissue mechanics inhibit constrictions parallel to the anterior-posterior axis of the embryo, so that apical cell surfaces primarily constrict along the dorsal-ventral axis to form a long, narrow furrow (Figure 2a) [27].

Surprisingly, instead of contracting in a uniform, steady progression, the medial myosin network in mesoderm cells contracts in brief pulses that are associated with bursts of myosin accumulation (Figure 2d) [22]. Cells undergo cycles of constriction and stabilization, which are translated into a net reduction in apical surface area by a ratchet-like mechanism that prevents complete relaxation of the cell surface and requires the Twist transcription factor [22].

Medial actin-myosin networks display similar pulsed contractile behaviors in the cells of the amnioserosa, a squamous epithelium on the dorsal surface of the *Drosophila* embryo (Figure 2b) [28-30]. Apical constriction of amnioserosa cells generates a force that pulls the lateral epidermis closed over the dorsal surface of the embryo. This process, together with

contraction of the leading edge cable [30-33], amnioserosa cell death [34], and filopodial protrusions [35,36], accomplishes dorsal closure.

Cycles of apical constriction and relaxation in the amnioserosa are associated with dynamic assembly and disassembly of the medial actin-myosin network [28,37]. Actin-myosin assembly and disassembly are proposed to be converted into sustained apical constriction by an external ratchet-like structure provided by a contractile actin-myosin cable at the leading edge of the adjacent lateral epidermis [30] or an intrinsic constriction program in the amnioserosa cells [37]. Dynamic actin-myosin foci have been observed in cortical meshworks in other cell types that display contractile behaviors, such as the one-cell *C. elegans* embryo [38] and intercalating mesenchymal cells in the *Xenopus* notochord [39]. Cycles of actin-myosin contractile activity may be a common property of highly dynamic, force-generating cells.

Contractile forces regulate cellular signaling and behavior

Mechanical forces have long been appreciated to play important roles in regulating cell shape, cell adhesion, cytoskeletal organization, and cell fate [40-43]. Polarized actin-myosin contractility initiated by centrosomal cues within the one-cell *C. elegans* embryo drives cortical flows that distinguish the anterior and posterior cortical domains [38,44,45]. This results in the asymmetric division of this cell into two blastomeres with different fates [46]. Ectopic and endogenous forces also regulate gene expression in the *Drosophila* embryo, where they promote expression of the Twist transcription factor [47,48]. The lineage of human mesenchymal stem cells is specified toward neurons on soft matrices that resemble brain tissue, myoblasts on matrices of intermediate stiffness, and osteoblasts on stiff matrices that resemble bone [43]. In addition, culturing human mammary epithelial cells in a matrix of elevated stiffness characteristic of mammary tumors disrupts epithelial morphogenesis by clustering integrins, which can contribute to malignant behavior [49].

Focal adhesions are large protein complexes that mechanically couple the interior of the cell to the extracellular matrix and constitute sites where external mechanical signals are transduced into biochemical signals [11,12,50]. The assembly and growth of focal adhesions are responsive to mechanical inputs [51,52]. Several components of cell-matrix adhesion have been shown to be directly regulated by force and may contribute to the force-dependent regulation of these processes [12,13]. Applying mechanical stretch to cellular cytoskeletal networks after extraction of the overlying membrane of mouse fibroblasts leads to activation of the small GTPase Rap1 [53], and biophysical studies show that force can directly expose a phosphorylation site for Src family kinases on the Src substrate p130Cas [54]. Other protein-protein interactions at focal adhesions that have been shown to be regulated by tension include fibronectin matrix assembly [55-57] and the association of talin with vinculin [58].

Emerging evidence indicates that force may play an analogous role in regulating adherens junctions that mediate cell-cell adhesion [10,59]. A recent study found that adherens junction size correlates with the magnitude of forces exerted between human epithelial cells in culture [60]. Vinculin, an essential component of cell-matrix adhesions, is also recruited to E-cadherin complexes under tension [61,62]. Vinculin has been proposed to bind to a cryptic site in the core adherens junction protein α-catenin that is exposed by mechanical stretch [62]. These results suggest a potential mechanism linking actin-myosin contraction to adherens junction stabilization during epithelial morphogenesis.

Mechanical cues regulate myosin dynamics

In addition to its role in generating the forces that shape tissues during development, recent studies show that myosin activity itself is also regulated by mechanical force. Myosin is recruited to the cortex in isolated *Dictyostelium* cells exposed to an ectopic force from a micropipette [63-65], a process that has been proposed to regulate cell shape in response to external deformation. In multicellular tissues, applying a force to the *Drosophila* embryo is sufficient to recruit myosin to the cortex during mesoderm invagination and axis elongation [66,67], while using laser ablation to relax tension locally leads to a rapid loss of myosin from the cortex [66]. These results demonstrate that tension is necessary and sufficient for myosin cortical localization.

During *Drosophila* axis elongation (Figure 3a), junctional myosin is localized to interfaces between anterior and posterior cells in the germband epithelium (Figure 3b), resulting in polarized cell rearrangements that elongate the body axis (Figure 3c) [68,69]. These cell rearrangements are driven by the contraction of single myosin-rich interfaces to promote local neighbor exchange [68] as well as the coordinated contraction of several connected cell boundaries to form multicellular rosette structures [70]. The anisotropic organization of myosin is associated with increased tension at interfaces between anterior and posterior cells [66,71], with the highest tension at the edges in multicellular contractile cables [66].

Fluorescence recovery after photobleaching experiments reveal that myosin dissociation from the cortex is selectively inhibited in regions of high tension [66]. In addition, multicellular actinmyosin cable formation in this tissue appears to be an active process that occurs at a higher frequency than expected by chance [66]. These results suggest a positive feedback loop in which the tension generated in one cell affects myosin dynamics in neighboring cells to promote multicellular contractile cable formation and efficient tissue elongation. Multicellular actinmyosin cables have been observed at the leading edge of the lateral epidermis during dorsal closure [31-33], at compartment boundaries [72-74], and during wound healing [75-78]. Mechanical signals may be important for the establishment and maintenance of these structures [79].

The recruitment of myosin by tension *in vivo* occurs within 1-3 minutes of applying force [64,66,67], suggesting that the response to force does not require transcription but instead involves a direct effect on myosin or its upstream regulators. In *Dictyostelium*, recruitment of the phosphoinositide phosphatase PTEN precedes myosin recruitment, and myosin accumulation in response to force is significantly reduced in *pten* mutants [80]. These results suggest that PTEN is part of a mechanosensory system that regulates myosin localization, perhaps by modifying properties of the plasma membrane. Consistent with this idea, the effects of applied tension in the *Drosophila* mesoderm are mimicked by inhibiting endocytosis [67].

Alternatively, myosin motor activity could also be directly regulated by force. During its ATPase cycle, non-muscle myosin II spends only a small fraction of the time attached to F-actin, resulting in a low duty ratio [5]. The binding of myosin to F-actin catalyzes phosphate release after ATP hydrolysis, which generates a strain in the motor that drives forward movement along the actin filament. Myosin remains strongly bound to F-actin after ATP hydrolysis, whereas subsequent ATP binding catalyzes the dissociation of myosin from F-actin. Single molecule measurements show that mechanical load stabilizes myosin II in the ADP-bound state when the motor is strongly bound to F-actin [81,82]. Indeed, in *Dictyostelium* the duty ratio of myosin was estimated to increase 5-10-fold under tension [65]. In the context of a contractile network, a fraction of myosin molecules will be attached to F-actin so that some motors actively pull on actin filaments while others mediate attachments between actin filaments. Increasing the duty ratio is predicted to increase the

fraction of motors bound to F-actin, which could potentiate the contractile activity of the network within an active range. Further work will be required to determine how tension-dependent changes in the myosin duty ratio influence network dynamics, contraction, and force generation.

Intrinsic properties of contractile actin-myosin networks

Several properties of the actin cytoskeleton have been reconstituted with purified proteins, providing an opportunity to investigate the molecular and physical mechanisms underlying the behavior of complex protein networks [83]. For example, the behavior of protrusive F-actin networks that drive the forward movement of migrating cells and propel bacterial pathogens through the cytoplasm can be recapitulated with a small number of components including actin, ATP, and proteins involved in actin filament nucleation, capping, and depolymerization [84-86]. These studies have provided a detailed understanding of how assembly and disassembly of the protrusive F-actin network generates force and movement.

Similarly, the *in vitro* reconstitution of actin-myosin networks has helped to elucidate physical principles that govern the dynamics of the contractile machinery. Processive assemblies of bipolar myosin filaments move actin filaments *in vitro* in two-dimensional filament gliding assays [87] and three-dimensional networks [88]. The ability of myosin to produce macroscopic network contraction depends on the concentration of actin crosslinking proteins [89,90]. In reconstituted systems containing only myosin, F-actin, and the actin crosslinking protein filamin, a minimum level of crosslinks is required for network contraction, while high concentrations inhibit contraction, presumably because the motors cannot generate sufficient force to contract the highly crosslinked network [90]. Because a fraction of myosin motors mediate attachments between actin filaments, contraction could in principle be varied by tuning the myosin duty ratio as well as the crosslink concentration.

Myosin motors can drive the formation of complex structures such as rings, asters, and active networks, depending on the relative concentrations of actin, myosin, and crosslinks [91]. High concentrations of myosin can also lead to the disassembly of these same structures [91], and myosin has been shown to promote actin depolymerization *in vitro* [92] and network disassembly in several cell types in culture [93-95]. These studies suggest that properties of cytoskeletal organization and dynamics can be intrinsically controlled by the molecular composition of contractile networks, a regulatory mechanism that may also influence cytoskeletal behavior *in vivo*.

Conclusions

The mechanical integration of forces generated by contractile actin-myosin networks and transmitted through cell-cell junctions is essential for the dynamic rearrangements that drive morphogenesis in actively remodeling cell populations. Here we describe several features of myosin networks revealed by studies *in vivo*, such as pulsed contractile behaviors during apical constriction and the recruitment of myosin by tension in single cells and multicellular tissues. It remains to be seen whether these behaviors represent intrinsic properties of contractile networks or if they are actively regulated by tissue-specific biochemical and mechanical cues. The transcription factors Snail and Twist are required to initiate pulses of contraction and stabilize the apical surface between contractions in the *Drosophila* mesoderm, respectively [22] and several targets of Twist are essential for mesoderm invagination [24,96,97]. In the amnioserosa, Baz/Par-3 stabilizes the actin-myosin network in a contracted state while Par-6 and aPKC regulate the interval between pulses [28]. Identifying the relevant transcriptional targets of Snail and Twist and the mechanisms by which Baz/Par-3, Par-6 and aPKC influence contractile behavior are challenges for future studies. Detailed investigation of the composition, architecture, and dynamics of cellular

contractile networks *in vivo* and comparison with the behavior of reconstituted networks *in vitro* will help to determine to what extent intrinsic properties are sufficient to account for network dynamics and force generation.

It is clear that properties of contractile networks influence the dynamics of mesoderm invagination, cell intercalation, and dorsal closure, but it is not known if these properties are essential for the ultimate outcome of morphogenesis. A mechanical feedback loop that recruits myosin to the cortex in regions of increased tension is predicted to increase the number of cells engaged in contractile behavior, converting local neighbor exchange events into multicellular rosette structures and producing greater tissue elongation during development [66]. This mechanism may also reinforce myosin activity in other multicellular contractile structures such as compartment boundaries [72-74,79]. The small, incremental changes afforded by pulsed contractile behavior during mesoderm invagination may allow the contractile machinery to adapt as cells change shape during tissue remodeling [22]. Transient or pulsed actin-myosin contractile behaviors are also observed in other contexts, such as in spreading and migrating mouse embryonic fibroblasts, where periodic myosin-dependent contractions of the lamellipodium may help to probe the local mechanical environment [98,99]. The combination of single molecule studies, reconstituted actin-myosin networks, and *in vivo* studies of myosin dynamics and cell behavior will uncover how intrinsic properties of contractile networks, together with the mechanical and biochemical cues that regulate cytoskeletal activity, contribute to cell shape and tissue morphogenesis during development.

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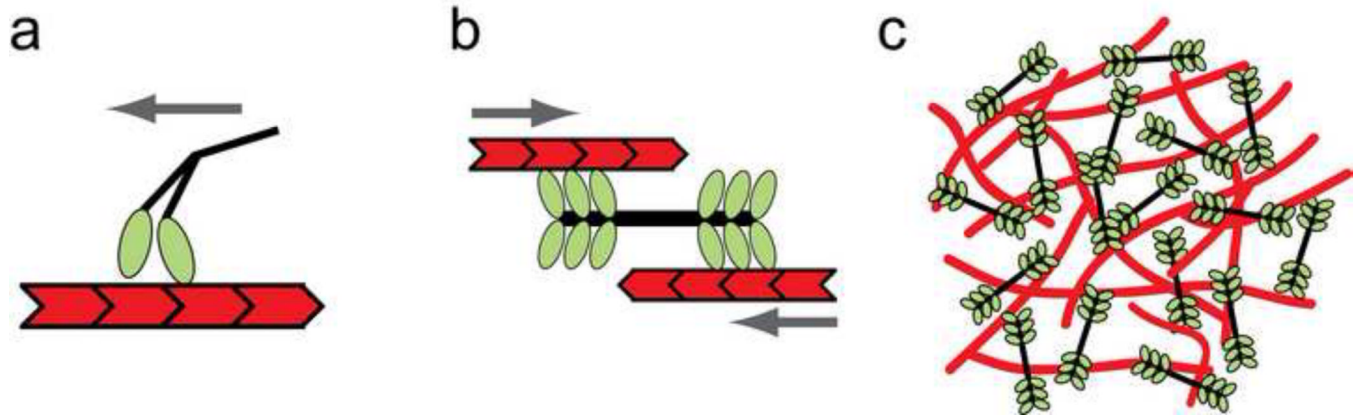

Figure 1. Contractile actin-myosin machinery

(a) A single non-muscle myosin II motor translocates toward the plus end of an actin filament (left). However, it has a low duty ratio and thus spends only a small fraction of its time bound to the actin filament. Because of this, the motor is non-processive and does not move continuously along the actin filament for long distances. Gray arrow indicates the direction of motor movement. (b) Several myosin motors can assemble into a processive, bipolar filament that generates relative movement between two anti-parallel actin filaments. Gray arrows indicate the direction of actin filament movement. (c) A contractile network formed from many actin filaments and bipolar myosin filaments. Myosin motor activity causes the network to contract.

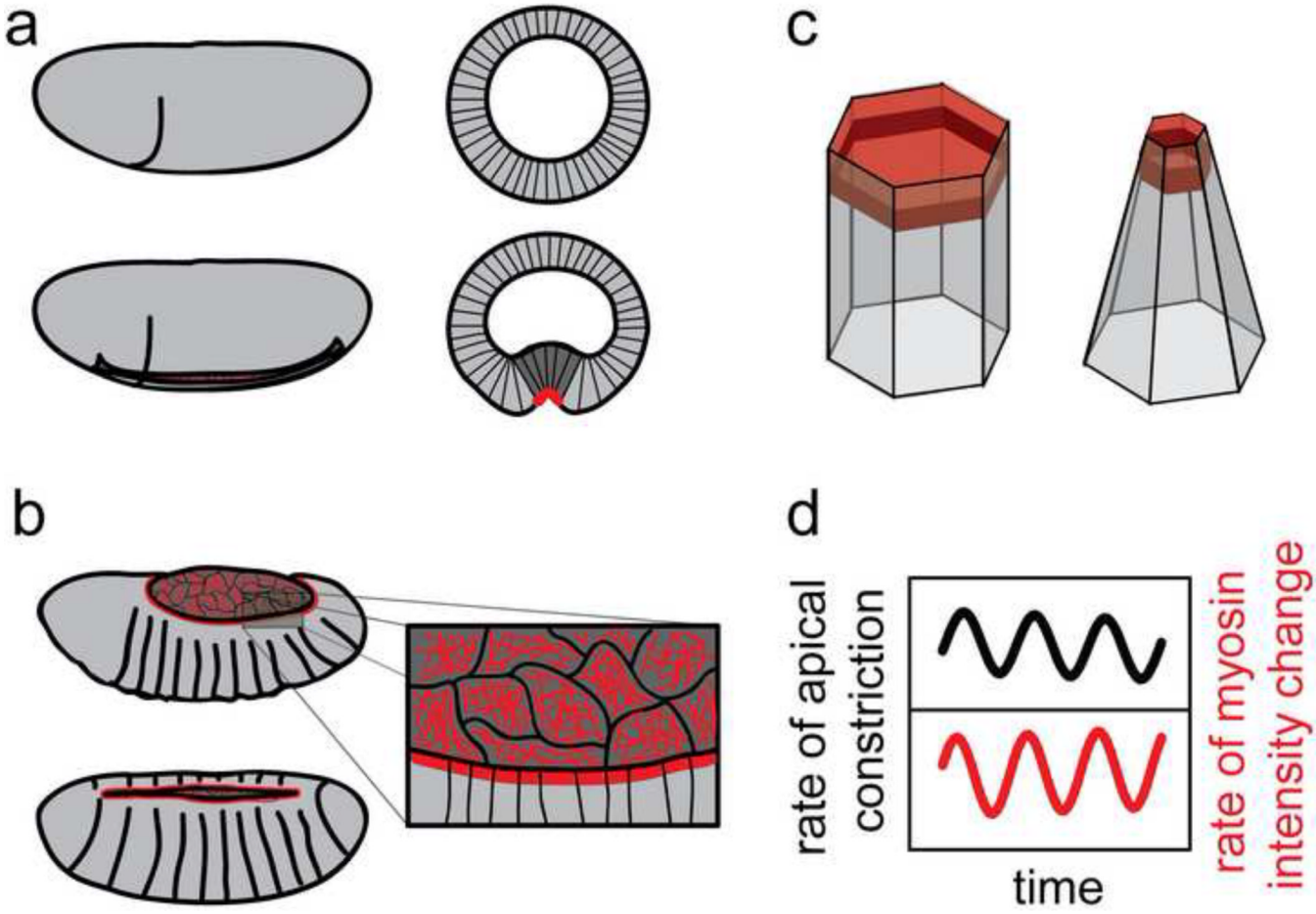

Figure 2. Pulsed contractile behaviors in apically constricting cells

(a) Prospective mesoderm cells on the ventral surface of the *Drosophila* embryo constrict their apical surfaces. This generates a bend in the tissue that causes the cells to invaginate to form a ventral furrow (*dark gray*). These cell shape changes are associated with an apical actin-myosin network (*red*). Before (*top*) and during (*bottom*) furrow formation. Lateral views, anterior left, ventral down (*left*), cross-sections (*right*). (b) Apical actin-myosin networks (*red*) also drive apical constriction of amnioserosa cells (*dark gray*), which generates one force that pulls the lateral epidermis closed over the dorsal surface of the *Drosophila* embryo. Contraction of the leading edge cable (*thick red line*), amnioserosa cell death, and filopodial protrusions also contribute to dorsal closure. (c) A medial actin-myosin network that spans the apical cell surface *(light red)* is connected through a second, junctional population that is anchored to adherens junctions at cell-cell contacts *(dark red)*. (d) Recent studies demonstrate that apical constriction occurs in brief pulses associated with fluctuations in the actin-myosin network. Apical constriction is closely correlated with bursts of myosin accumulation.

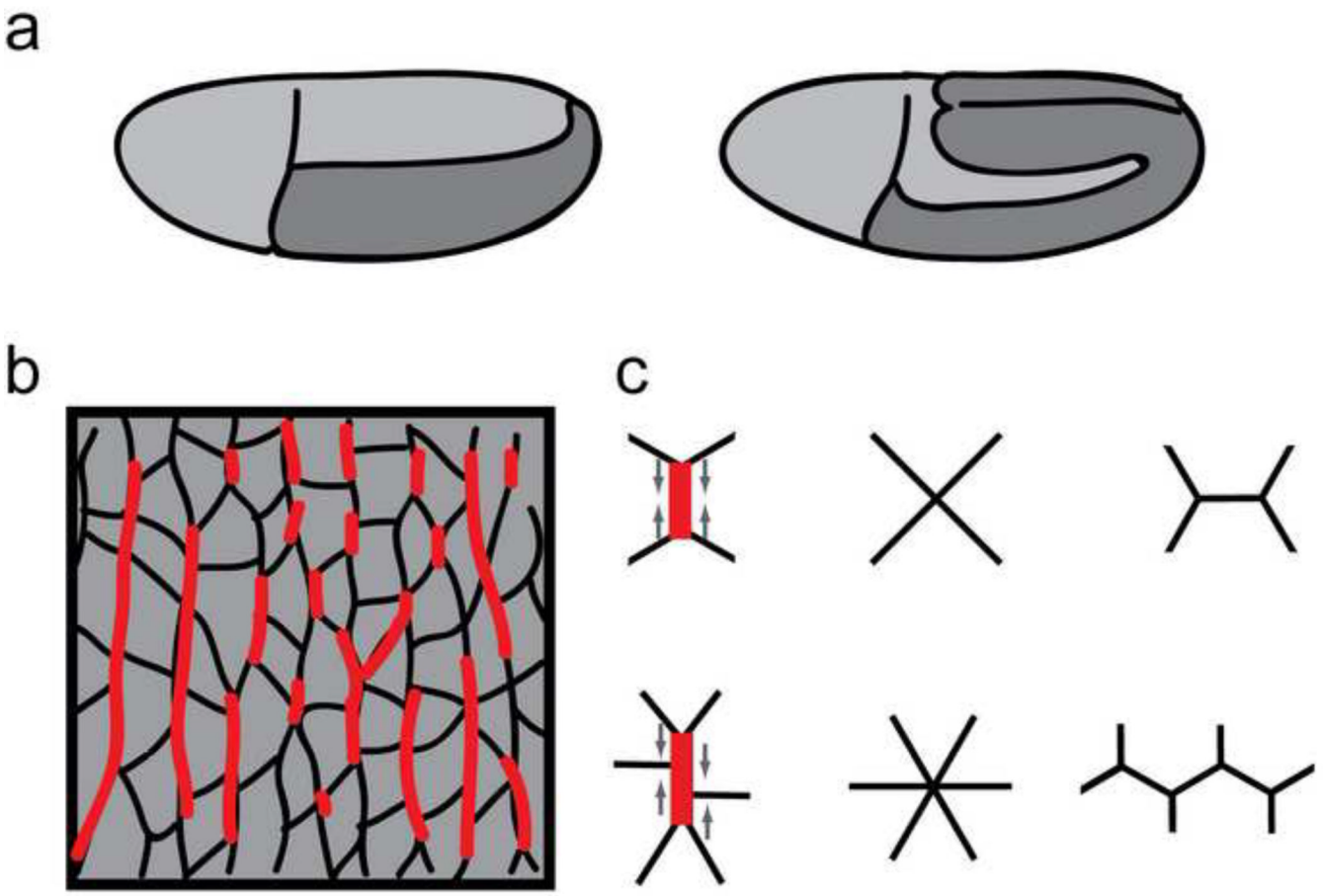

Figure 3. Role of actin-myosin in elongation of the *Drosophila* body axis

(a) The germband epithelium (*dark gray*) lengthens and narrows to elongate the body axis. Before (*left*) and after (*right*) elongation. Lateral views, anterior left, ventral down. (b) Junctional myosin (*red*) is localized to vertical interfaces between anterior and posterior cells, including single cell interfaces and multicellular cables. Laser ablation experiments reveal that myosin-rich interfaces are under tension, with the highest tension in multicellular cables. (c) Polarized cell rearrangements contribute to elongation of the body axis. Contraction of a single myosin-rich interface promotes local neighbor exchange (*top*), and the coordinated contraction of several adjacent cell interfaces forms a multicellular rosette structure that promotes many-cell rearrangements (*bottom*). Mechanical tension promotes multicellular cable formation, recruiting myosin to the cortex in regions under high tension.