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Review

Principles of Actomyosin Regulation *In Vivo*

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The actomyosin cytoskeleton is responsible for most force-driven processes in cells and tissues. How it assembles into the necessary structures at the right time and place is an important question. Here, we focus on molecular mechanisms of actomyosin regulation recently elucidated in animal models, and highlight several common principles that emerge. The architecture of the actomyosin network – an important determinant of its function – results from actin polymerization, crosslinking and turnover, localized myosin activation, and contractility-driven self-organization. Spatiotemporal regulation is achieved by tissue-specific expression and subcellular localization of Rho GTPase regulators. Subcellular anchor points of actomyosin structures control the outcome of their contraction, and molecular feedback mechanisms dictate whether they are transient, cyclic, or persistent.

Highlights

Actomyosin contractility, which shapes cells and tissues during development, wound repair, and physiology, is regulated by the actin network architecture, connectivity and turnover, as well as myosin-II dependent self-organization.

Spatiotemporal regulation of contractility results from localized and timely activation of Rho signaling downstream of cellular polarity regulators, or membrane receptors and tissue-specific transcription factors.

Anchoring of actomyosin structures at cell–cell and cell–extracellular matrix adhesions is important for force transmission, as well as regulation of contractility. Mechanical feedback from adhesions on actomyosin regulates developmental processes.

Feedback loops generating cycles of Rho activation and inactivation are responsible for pulsatile contractile behaviors that are prevalent in animal development and physiology.

Diverse Actomyosin Structures Drive a Variety of Processes at the Subcellular, Cellular, and Tissue Level

Actin and non-muscle myosin-II combine to form actomyosin networks capable of generating contractile forces. Animal cells use these forces in a wide variety of processes that range in scale from the subcellular to the tissue level, and can be transient or sustained. Examples of contractions at the subcellular level are retraction of plasma membrane blebs [1] and clatherin-mediated endocytosis [2]. Contractions at the cell scale include cortical polarization [3], cytokinesis [4], and fast amoeboid cell migration [5,6]; and at the tissue level contractions mediate **convergent extension** (see Glossary) [7], gastrulation [8,9], and support syncytial germline architecture [10], to name but a few. The contractile machineries involved in all these processes are comprised of the same core components, namely actin and non-muscle myosin-II, but they differ in their size, location, auxiliary components, internal organization, and connection with other cellular structures. This raises an important question: how are the necessary actomyosin structures assembled at the correct time and place to drive cell and tissue morphogenesis, and sustain their architecture and physiology? Studies over the past four decades provided us with substantial understanding of the molecular mechanisms controlling actomyosin contractility in cultured cells and in cell-free systems. However, in recent years, advances in imaging techniques, and a growing appreciation for the role of mechanical forces in biology, has led to a surge in studies examining actomyosin contractility in animal models, shedding light on its more complex regulation *in vivo*. While the emerging picture is far from complete, in this review we attempt to outline some of the emerging principles of actomyosin regulation *in vivo*. Knowledge of these principles is essential to understanding embryonic development and can also guide tissue engineering approaches.

Actomyosin Network Architecture Controls Its Function

The basics of actin and myosin are described in Box 1. In contrast with muscle cells, in which actin and myosin are arranged in highly ordered and stable sarcomeres, actomyosin in non-muscle cells has more versatile arrangements, including bundles and 2D networks.

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Box 1. Basics of Actomyosin Contractility

The fundamental biophysical properties of actin and myosin, namely that globular actin polymerizes into polar filaments (F-actin), that myosin II motors can only 'walk' along F-actin toward the plus end (Figure I), and that myosin II mini-filaments have motors pointing in two opposite directions, explain why the relative orientation of F-actin and myosin II mini-filaments is paramount in determining the outcome of ATPase-fueled myosin motor activity. Along parallel actin filaments, myosin moves without displacing the actin, whereas anti-parallel actin filaments will be displaced relative to each other (and could, in theory, generate contractile or extensile forces). In reality, extensile forces are never observed, possibly because of bending and buckling of actin filaments [34] and/or because myosin mini-filaments rotate into

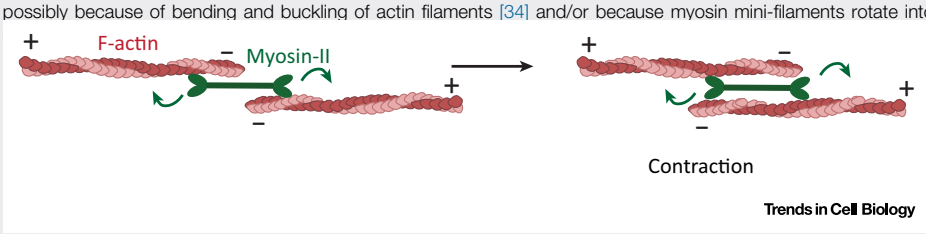

Figure I. Generation of Contractility. Sliding of antiparallel actin filaments (red) caused by the movement of myosin motors (green) towards the actin plus ends, generates contraction.

Bundles can be linear or form rings. Actomyosin rings anchored to the plasma membrane are found in dividing cells [4] (Figure 1Ai), and in cells being extruded from a monolayer [11]. Multicellular rings, made up of segments contributed by individual cells, anchored to and coordinated by their cell–cell junctions, are used in a 'purse string' fashion to close epithelial holes formed during developmental processes, such as *Drosophila* dorsal closure (Figure 1Aii) and wound healing (reviewed in [12]). In most cases, the internal organization of actomyosin bundles is not discernable in light microscopy. However, periodic assemblies of myosin mini-filaments interspersed with perijunctional actin and α-actinin have been documented in the mouse organ of Corti, where these sarcomere-like structures form a continuous belt around each epithelial cell [13] (Figure 1Aiii). A similar 'bars-on-a-string' organization of myosin-II alternating with α-actinin has been observed at the leading edge of epidermal cells during dorsal closure in flies [14,15]. In contrast, actin and myosin display stochastic patterns around embryonic wounds, and heterogeneous contractile forces favor wound closure [16].

Linear cables are found along cell–cell junctions at the border of tissues that are not meant to mix, as seen in between anterior and posterior compartments of the wing disc of *Drosophila* (Figure 1Bi) [17,18]. They also help in rearranging epithelia, such as during convergent extension. Accumulation of actomyosin along a row of interfaces leads to their contraction and shortening along one axis, followed by elongation along the orthogonal axis, causing tissue elongation. This process of **cell intercalation** and convergent extension results in germ band extension in the *Drosophila* embryo [19] (Figure 1Bii), kidney tubule extension in mouse and *Xenopus* [20], and neural tube formation in the chick [21]. Contractility of the *Caenorhabditis elegans* spermatheca, a tubular myoepithelial tissue involved in reproduction, is driven by arrays of basally localized circumferential actomyosin bundles [22] (Figure 1Biii).

Two-dimensional actomyosin networks form the cortex underlying the plasma membrane of all animal cells [23]. In *Drosophila* embryonic epithelial cells undergoing apical constriction during ventral furrow formation, medioapical actomyosin is organized in a polarized radial network, with myosin in the center, and barbed ends of actin anchored at the periphery of the cell, generating a sarcomere-like periodicity at the tissue scale. Loss of this polarized network organization inhibits apical cell contractility and tissue folding [24] (Figure 1Ci). In the *C. elegans* zygote cortex, contracting myosin coalesces into visible foci, which remain interconnected by

Glossary

Adherens junctions: this is one of the several types of cell–cell adhesion structure required for intercellular contact. Major structural components of adherens junctions include transmembrane protein cadherins, catenins and cytoskeletal adapter proteins.
Cell intercalation: this is a process of exchange of neighboring cells within a tissue observed during gastrulation in several organisms.
Convergent extension: this is a process in which cells migrate and rearrange to cause narrowing and lengthening of the tissue during development.
Crosslinker: actin binding proteins that organize actin filaments into complex higher order network.
Cytohesins: belong to family of guanine nucleotide-exchange proteins (GEPs) required for the activation of ADP-ribosylation factors (ARFs).
Cytokinetic furrow: a small ingression formed at the cell cortex at the beginning of cell division caused due to pulling of actomyosin ring.
E-cadherin: is a classical cadherin (calcium-dependent cell adhesion molecule) present at cell junctions in the epithelial tissue. The extracellular domain of E-cadherin interacts with E-cadherin present on the adjacent cells while the intracellular cytoplasmic domain interacts with actin cytoskeleton.
GAPs: GTPase activating proteins (GAPs) inactivate GTPase by catalyzing hydrolysis of GTP to GDP.
GEFs: guanine nucleotide exchange factors (GEFs) are the proteins that activate GTPase by promoting the dissociation of GDP in exchange of GTP.
GPCR: G protein-coupled receptors (GPCRs) are the seven transmembrane containing proteins that regulate several cellular processes. Ligand binding activates GPCRs, which then undergoes conformational changes to bind intracellular heterotrimeric G protein. Active G proteins lead to the activation of multiple downstream signaling cascades.
Pair-rule transcription factors: these are the components of

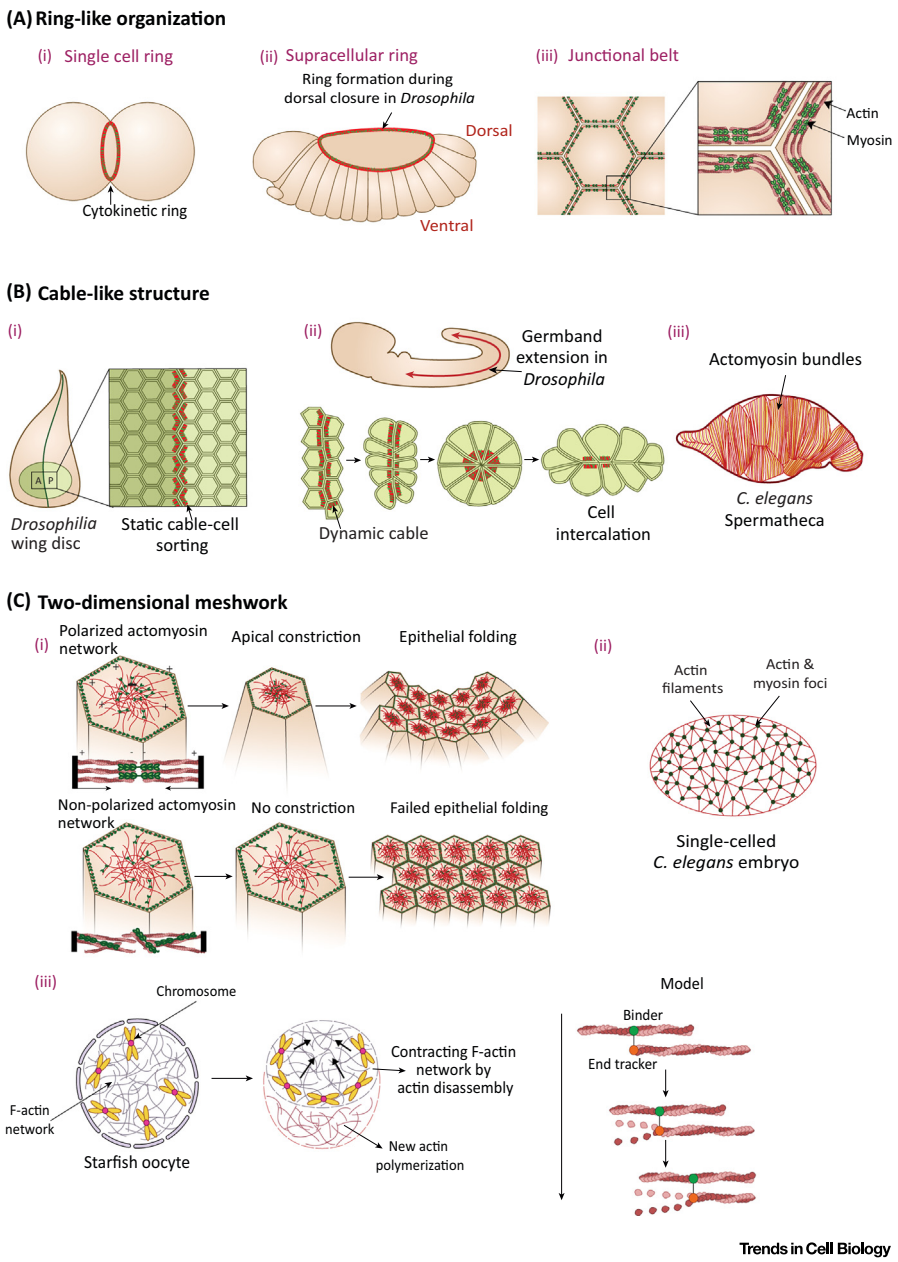

Figure 1. Diverse Actomyosin Structures Observed *In Vivo*. (A) Ring-like organization. (i) Single-cell actomyosin ring formed at the equatorial region of a dividing cell. (ii) A supracellular contractile ring-like structure spanning several cells during dorsal closure in *Drosophila* embryo. (iii) A belt of actomyosin network (sarcomere-like arrangement in the inset) present at the junctions of epithelial cells of organ of Corti of mouse. (B) Cable-like structures. (i) Static cable (red and green) prevents cell mixing and allows the formation of a boundary between different cell fates of the anterior (A) and posterior (P) compartments in the *Drosophila* wing disc. (ii) Dynamic cable contracts to form a rosette that allows cell intercalation and axis elongation during germ-band extension in *Drosophila*. (iii) Actomyosin bundles in the *Caenorhabditis elegans* spermatheca. (C) Two-dimensional meshwork. (i) Mesoderm cells in *Drosophila* have a polarized medio-apical actomyosin network, plus ends of the actin filaments directed towards the junction and minus ends towards the center, which constrict cells apically to cause tissue folding during ventral furrow formation. Loss of polarity prevents apical constriction and

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anterior-posterior patterning during *Drosophila* embryogenesis.
Pulse-assembly: assembly of actomyosin machinery that leads to contraction followed by rapid disassembly of the contractile machinery.

actin cables [25] (Figure 1Cii). Relaxation of cortical contractility at the site of sperm entry, triggers a polarized flow of the cortex that initiates zygote polarization for asymmetric cell division [26]. A unique three-dimensional actomyosin structure was recently found within the syncytial germline of *C. elegans*. Contractility of this inner corset-like actomyosin structure is tightly regulated to support germline architecture and function [10].

Actin Polymerization, Connectivity, and Turnover Are Required for Effective Contractility

Actin polymerization in cells is catalyzed by several nucleation and elongation factors, most notably the arp2/3 complex, which forms branched F-actin networks, and formins and Ena/VASP proteins, which form linear arrays of F-actin (reviewed in [27]). While each nucleator can function independently, many actomyosin networks depend on the cooperative activities of multiple actin assembly-promoting factors. For example, assembly of the cytokinetic ring in *C. elegans* appears to require only the formin CYK-1 [28], whereas the formation of an actomyosin coat around glue-protein vesicles in the salivary gland of *Drosophila* requires activity of both the formin Dia and arp2/3 [29].

Crosslinking of F-actin by specialized proteins, such as α-actinin, as well as myosin mini-filaments themselves, is essential to increase the length, scale, and effectiveness of actomyosin network contraction. However, too much crosslinking activity will rigidify the network and inhibit contraction. This was shown in theory and in *in vitro* experiments [30], but only recently it was shown to be the case *in vivo*. Plastin, a major **crosslinker** in the cortex of the *C. elegans* zygote, is required at just the right amount, to enhance connectivity and enable the cortical rearrangements that drive polarization and cytokinesis [25]. Plastin is also required for the organization of cortical actomyosin network in the mouse epidermis [31]. Similarly, the maximal rate of contraction of the **cytokinetic furrow** is achieved at intermediate levels of the scaffold protein Anillin [32].

F-actin turnover, enhanced by the activity of actin depolymerizing factors, such as cofilin, also plays a major role in regulating contractility. In contrast with muscle sarcomeres, in which actin filaments are very stable, actomyosin networks in non-muscle cells are extremely dynamic. Without severing and depolymerization of F-actin, the network will get stuck. For instance, vesicle constriction during exocytosis of glue protein into the lumen of *Drosophila* salivary glands requires a continuous cycle of F-actin assembly and turnover. Stabilizing F-actin by treatment with jasplakinolide halts this contraction [33].

Moreover, theory and simulations predict that F-actin turnover in a disordered actomyosin network will generate a pulsatile behavior [34,35]. Pulsatile contractility has been observed *in vivo* in several models, including *Drosophila* amnioserosa cells during dorsal closure, the *C. elegans* zygote cortex during polarization, and *Xenopus* mesoderm during convergent extension [36]. A pulsatile medioapical actomyosin network in *Drosophila* epithelial cells drives apical constriction that results in gastrulation only if it is continuously anchored at **adherens junctions**, and this connection was shown to depend on F-actin turnover [37].

Besides facilitating actomyosin pulsation, F-actin turnover has been suggested, in some cases, to drive myosin-II-independent contraction of an F-actin network. Budding yeast cytokinesis

epithelial folding. (ii) Actomyosin meshwork in the cortex of the *C. elegans* embryo. (iii) F-actin disassembly-mediated contraction of the actomyosin network present in starfish oocytes transports chromosomes towards the spindle. A model proposes that the contractile force is generated by a crosslinker, which tracks the depolymerizing actin filament at one end (end tracker-orange), while the other end (binder-green) remains attached to an adjacent actin filament.

and starfish oocyte chromosome transport have both been proposed to be based on a putative end-tracking F-actin crosslinker that harnesses F-actin depolymerization for the contraction of a ring or sphere of F-actin, respectively [38,39] (Figure 1Ciii). Probably, a similar mechanism operates for myosin-II independent actin dynamics during neurulation in mouse embryos. Neural tube closure is blocked by depleting the actin-severing protein, cofilin, or treatment with the F-actin stabilizer, jasplakinolide, but remains unaffected after treatment with myosin inhibitors like blebbistatin and ML-7 [40]. Interestingly, *Drosophila* cellularization has been proposed to be driven sequentially by myosin-dependent and myosin-independent contraction mechanisms [41].

Self-Organization of Actomyosin Networks

The architecture of actomyosin networks can be further modified by the activity of myosin-II itself. In cultured fibroblasts, it was found that myosin-II motor activity is essential for parallel stress fibers to align themselves such that their myosins form orderly stacks [42]. A similar phenomenon has been observed *in vivo* in the nematode reproductive system. Prior to the first ovulation, the actomyosin network present in the *C. elegans* spermatheca appears tortuous, branching, and randomly oriented. However, after the first ovulation, prominent, parallel actomyosin bundles aligned along the long axis of each cell were apparent, and their formation was shown to depend on myosin-II activity [22]. In *Drosophila* tracheal tubules, actin filaments at the apical cortex were initially uniform. However, later in development, concurrent with, and dependent on an increase in actomyosin activity, the actin reorganizes to form a regular pattern of 15–20 circumferential actin rings per cell [43]. Another case where the subcellular position of an actomyosin structure is affected by myosin activity is in *Ciona intestinalis* embryogenesis. The opposing forces generated by myosin contractility and polarity regulators position an actomyosin ring at the equator of the notochord cells, which is essential for cell elongation [44].

Spatiotemporal Activation of Actomyosin

Spatial and temporal regulation of actomyosin contractility is achieved by a combination of tissue-specific expression of different transcription factors, and subcellular signaling pathways, such as apicobasal or planar polarity. For example, tissue elongation is caused by planar polarized localization and activation of myosin-II that depends upon the presence of Toll receptors activated by the **pair-rule transcription factors**, Even-skipped and Runt in *Drosophila* [45], and planar polarity regulator Ptk7 in the mouse neural plate [46]. Apical constriction during tissue folding is triggered by the enrichment of actomyosin at the apical domain of cells via localized expression of the G protein-coupled receptor (**GPCR**) Mist, activated by the transcription factor Snail in *Drosophila* [47] and via the actin-binding protein Shroom3, transcriptionally regulated by Pitx, in the *Xenopus* gut [48]. Furthermore, a common mediator can drive tissue-specific localization of myosin-II in distinct regions within the same embryo by interacting with tissue-specific ligands and/or another mediator. Smog, a ubiquitously expressed GPCR protein, regulates medio-apical activation of myosin-II during mesoderm invagination in *Drosophila* embryos, while it polarizes contractility at the junctions during ectoderm extension. Smog interacts with the mesoderm-specific ligand Fog and a mesoderm-specific GPCR, Mist, to activate apical myosin-II in the mesoderm, whereas the modulators acting with Smog in the ectoderm are still unknown [49]. These studies show how diverse tissue-specific signals demarcate the subcellular location of contractility.

Rho GTPases Are Major Regulators of Contractility *In Vivo*

Many of the tissue-specific regulators of contractility discussed above interact directly or indirectly with components of the well conserved Rho GTPase signaling pathway, including

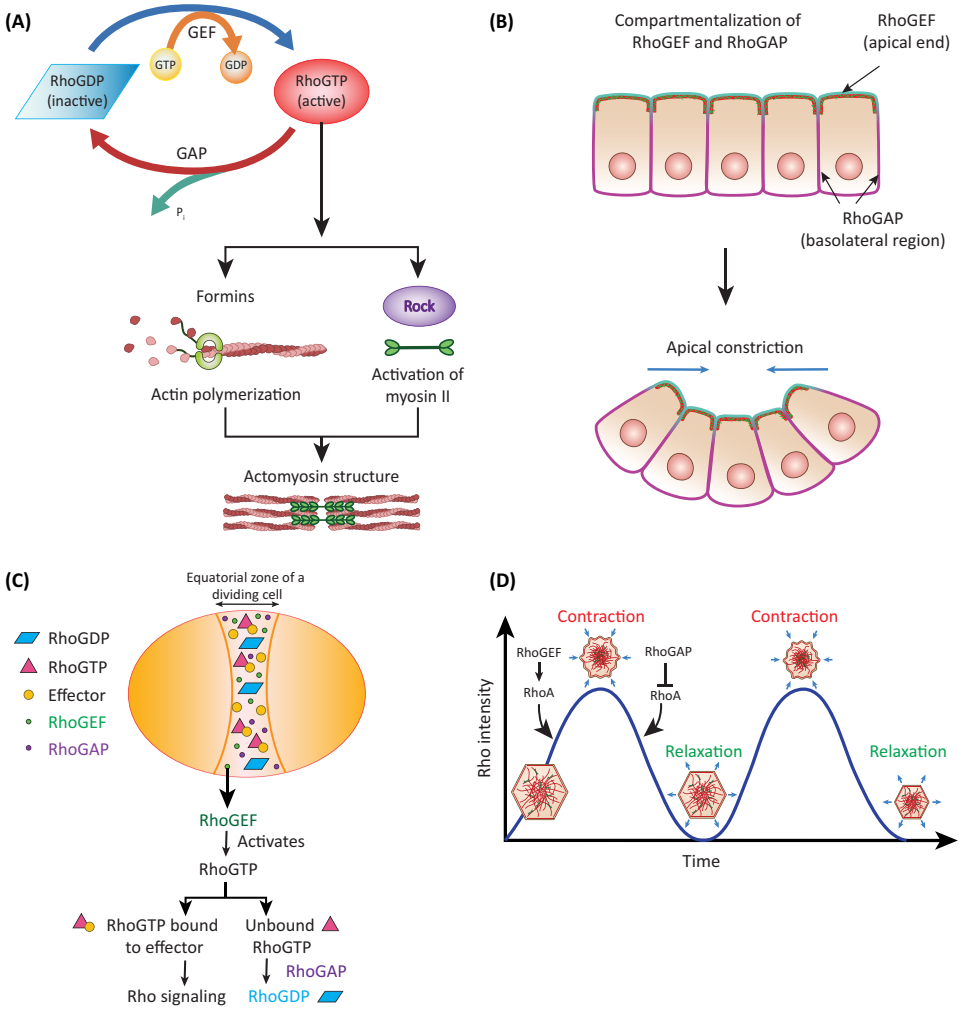

Figure 2. Spatiotemporal Regulation of Contractility by Rho Signaling. (A) Schematic of Rho signaling pathway. The activation of Rho GTPase is catalyzed by RhoGEFs (guanine nucleotide exchange factors), which exchange GDP with GTP, and their inactivation is catalyzed by RhoGAPs (GTPase activating proteins). In its active form, RhoGTP binds to its effectors, formins, and Rho kinase (ROCK), which polymerize actin and phosphorylate and activate myosin-II, respectively, leading to the formation of actomyosin structures. (B) Spatial restriction of Rho activity at the apical side of the cell due to differential localization of RhoGEFs (cyan) at the apical surface, and RhoGAPs (magenta) at the basolateral surface, leads to apical constriction and tissue folding. (C) A focused zone of Rho activity, at the equator of a dividing *Xenopus* embryo, is maintained by a balance between localized Rho activation, and rapid Rho inactivation. Activated Rho GTPase (by RhoGEF) binds to an effector (within a restricted zone) to activate downstream components of Rho signaling, otherwise it gets rapidly inactivated by RhoGAPs. (D) Temporal regulation of contractility. Pulses of contraction and relaxation of the actomyosin network during apical constriction are driven by the cycles of RhoA activation mediated by RhoGEF and RhoA inactivation mediated by RhoGAP.

Rho, Rac, and Cdc-42 [50]. Rho GTPases regulate actomyosin contractility by promoting polymerization of actin via formins or arp2/3, and/or by promoting myosin light chain (MLC) phosphorylation by activating the myosin light chain kinases MRCK (myotonic dystrophy kinase-related Cdc-42-binding kinase) or Rho kinase (ROCK), which also inhibits myosin phosphatase (MLCP) [51] (Figure 2A).

Rho GTPases cycle between a GTP bound 'active' form and a GDP bound 'inactive' form. Inactive Rho::GDP can be made active by Rho**GEFs** (guanine nucleotide exchange factors), and Rho::GTP can be rendered inactive by Rho**GAPs** (GTPase activating proteins) [51]. Distinct localization of GEFs and GAPs within a cell restricts Rho signaling to a specific region. For example, apical constriction of the enfolding epithelial cells during spiracle formation in *Drosophila* is caused by the localization of Rho activators – RhoGEF2 and RhoGEF64C – at the apical end, and Rho suppressor – RhoGAP Crossveinless-c – at the basolateral surface [52] (Figure 2B). In early *C. elegans* embryos, Cdc-42 is inactivated by the RhoGAP PAC-1, which is recruited by an E-cadherin-p-120-catenin-PICC-1 complex at surfaces in contact with other cells [53]. Active CDC-42 at the contactless apical surface is then free to activate MRCK, which phosphorylates MLC to initiate gastrulation [54].

A GTPase flux model has been proposed for creating a spatially constrained Rho active zone when GEFs and GAPs are present within the same region [55]. According to this model, Rho GTPases undergo cycles of activation and inactivation (GTPase flux) by GEFs and GAPs, respectively, inside a specific zone. Upon activation by a RhoGEF, Rho::GTP has a short-lived opportunity to bind to an effector, which activates downstream signaling, otherwise it will rapidly be inactivated by a RhoGAP. This prevents the movement of active Rho GTPase outside the specific zone, creating a confined region of active Rho signaling [55] (Figure 2C). Consistent with this model, depletion of GAP proteins like MgcRacGAP in *Xenopus* embryos, and M-phase RhoGAP along with centrosome asters in *C. elegans* embryos, results in an unfocused zone of active RhoA impairing the process of cytokinesis [56,57]. Also, a colocalized alternating cycle of RhoGEFs and RhoGAPs can cause dynamic pulsatile contractile activity, as observed during ventral furrow formation in flies and in the cortex of a dividing single-celled *C. elegans* embryo [58,59] (Figure 2D).

The complexity of regulation by Rho signaling is further increased by the presence of multiple RhoGEFs and RhoGAPs that act in cooperation or antagonistically with each other to regulate specific developmental processes. For example, RhoGEF ECT-2 and RhoGAP CYK-4 activate RHO-1 at the furrow region for cytokinesis [60,61], while ECT-2 and the RhoGAPs RGA-3/4 act antagonistically to stabilize the position of the cytokinetic furrow in *C. elegans* embryos [62]. Multiple RhoGEFs coordinate with each other to pattern the localization of Rho GTPases – Rho-1, Rac, and Cdc-42 during wound healing in flies [63].

Anchoring Points of Actomyosin Networks Determine the Outcome of Their Contraction

Actomyosin structures do not 'stand alone' in the cell but rather are connected either to a membrane, or to the nucleus, and/or to sites of cell–matrix or cell–cell adhesion. The outcome of contractility of a given actomyosin structure depends on its anchor points within the cell. For example, anchoring of the circumferential actomyosin belt at adherens junctions will generate sustained tension at cell–cell junctions to maintain epithelial tissue tautness [64]. The actomyosin cables formed in response to epithelial wounding are mechanically integrated into a ring through coordinated anchoring at adherens junctions [65]. Also, the linear cables formed downstream of planar polarity, which are responsible for convergence and extension, are anchored at cell–cell junctions. The cortex is an actomyosin network that is linked to the plasma membrane, but not anchored at junctions. Its contraction can facilitate clustering of membrane bound proteins, such as the polarity regulator PAR-3 [3]. Anchoring of contractile bundles at cell–matrix adhesions promotes efficient force-transmission across the tissue essential for morphogenetic events, such as dorsal closure in *Drosophila*, and retinal tissue folding in *Zebrafish*. Genetically depleting or enhancing cell–ECM adhesion impedes these processes [66,67].

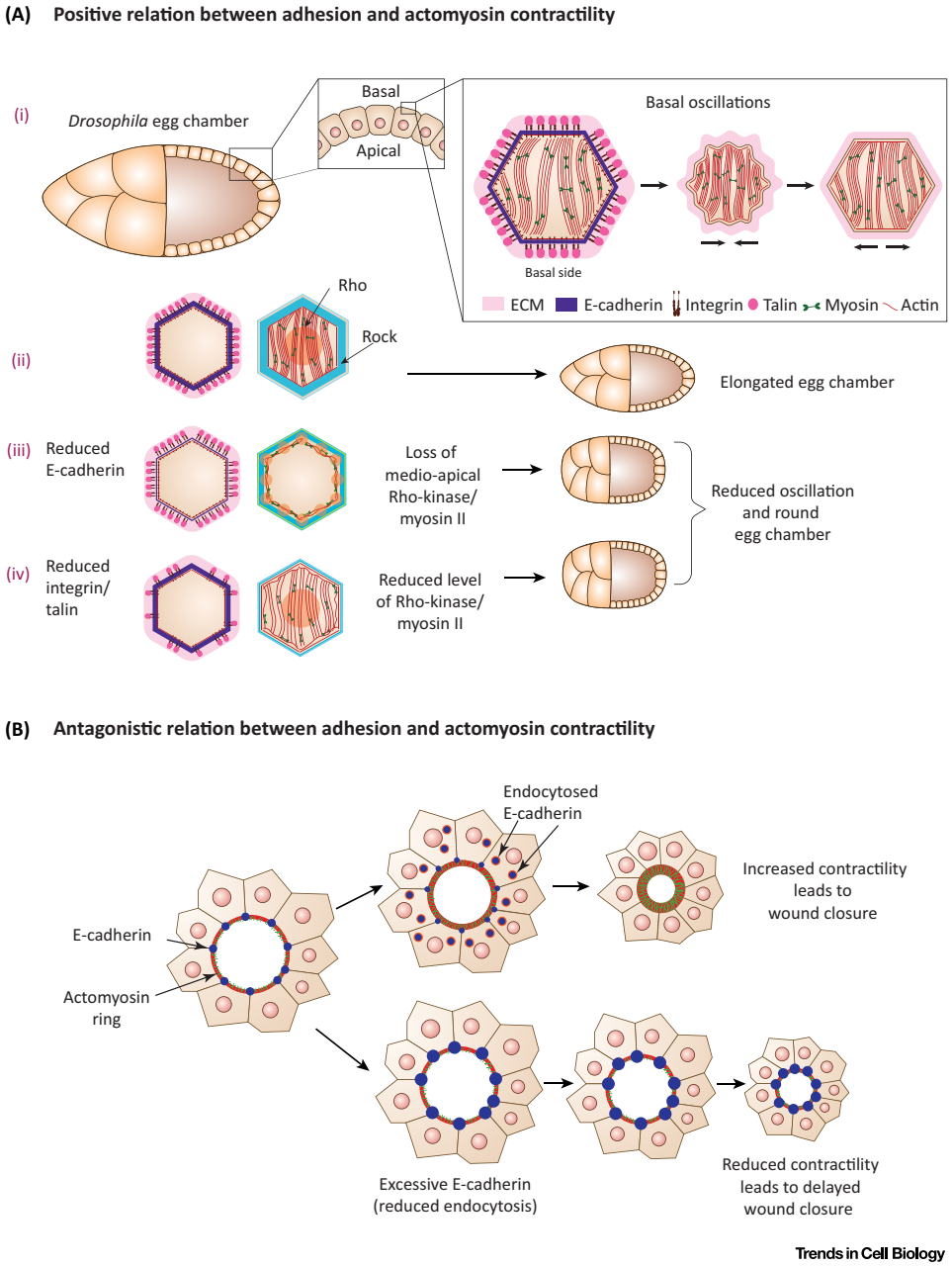

Figure 3. Contextual Relationship between Cell Adhesion and Contractility. (A) Positive relation between adhesion and actomyosin contractility. (i) Schematic diagram of a stage-10 egg chamber of *Drosophila* showing the localization of extracellular matrix (ECM), E-cadherin, integrin, talin, actin, and myosin at the basal surface of the follicle cells. Myosin-II oscillations at the basal domain of the follicle cells (enlarged inset) regulate egg chamber elongation. (ii) Distribution pattern of E-cadherin (dark blue), Rho (orange), Rho kinase (ROCK) (light blue), and actomyosin (red fibers at center) at the basal surface (shown in enlarged inset) of the follicles required for egg chamber elongation. (iii and iv) Reduced E-cadherin or talin/integrin disrupts the localization and activity of ROCK or myosin-II, respectively, inhibiting basal myosin-II oscillations and egg chamber elongation. (B) Antagonistic relation between adhesion and actomyosin contractility. During epithelial wound repair, E-cadherin (blue) is down-regulated via polarized endocytosis to enable assembly of a supracellular actomyosin ring (red and green) essential for rapid wound closure. Overexpression of E-cadherin (blue) (via inhibition of endocytosis) results in reduced accumulation of actin and myosin (red and green) at the wound site, and hence, a significantly slower rate of wound healing.

In addition to providing anchoring points through which contractile forces are transmitted to other tissues, cell–cell and cell–matrix adhesion sites also actively regulate actomyosin contractility. During *Drosophila* egg chamber elongation, basal cell contractions are regulated by cell–matrix adhesion and cell–cell adhesion in two distinct ways: cell–cell adhesion via **E-cadherin** restricts the localization of myosin-II at the medio-basal region, while cell–matrix adhesion mediated via integrins and talin is required for the activity or level of Rho and myosin-II at the basal side (Figure 3Ai,ii). Depleting cell–cell or cell–matrix interaction disrupts the distribution pattern or level of myosin-II, respectively, leading to reduced basal cell contractions which prevents egg chamber elongation [68] (Figure 3Aiii,iv). Similarly, interaction of the cell cortex with cell–ECM adhesion complexes, patterns the formation of actin rings at regular intervals in the tracheal tube of *Drosophila* [43].

Contrary to the above instances where adhesion is required for efficient actomyosin contractility, an antagonistic relationship between cell–cell adhesion and contractility is observed in multiple cellular processes. Elimination of E-cadherin by endocytosis at the site of an embryonic wound allows the assembly of an actomyosin cytoskeleton belt crucial for wound closure. An excess of E-cadherin, caused by blocking endocytosis, prevents the accumulation of actomyosin machinery, and delays wound healing [69,70] (Figure 3B). E-cadherin and myosin-II are also mutually exclusive in the early *C. elegans* embryo, and knockdown of E-cadherin leads to an increase in cortical myosin-II [71]. Remarkably, these effects are not limited to cadherin engaged in cell–cell contacts. Non-junctional E-cadherin clusters impede cortical flows, and slow down furrow ingression [71]. In *Drosophila*, tension generated by the cytokinetic ring elongates adherens junctions at the site furrow ingression. This results in the dilution of E-cadherin, causing localized enrichment of myosin-II essential for disengagement with adjoining cells, and junctional remodeling for the formation of new contacts [72–74]. Finally, both positive and negative regulation of contractility via N-cadherin at heterotypic and homotypic cell contacts, respectively, control the formation of proper cell shape and arrangement in the *Drosophila* eye [75].

Cell and Tissue Level Mechanical Feedback Mechanisms Regulate Contractility

Feedback regulation is an efficient way to make a system robust and self-organizing. Positive feedback ensures a continuous presence of signal, making the process self-sustained, while negative feedback ensures the transience of a signal. Feedback regulation between mechanical signals and actomyosin contractility orchestrate several events of tissue morphogenesis and maintain homeostasis. Cytokinetic ring constriction is driven by a positive feedback loop between the myosin at the ring and cortical flow [76]. The constricting ring pulls its adjacent cortex, causing a flow of myosin towards the ring that results in higher levels of myosin, and hence, amplified constriction rate per unit length with decreasing perimeter [76]. In response to the pulling force by the cytokinetic ring, there is also increased recruitment of myosin-II at adherens junctions, which promotes junctional remodeling between neighboring and dividing cells [73] (Figure 4A).

Negative feedback regulation of actomyosin contractility at adherens junctions helps to relieve mechanical stress during tissue elongation in *Drosophila* and *Zebrafish* [77]. In the time scale of minutes, actomyosin enrichment recruits Steppke, an Arf-GEF of the **cytohesin** protein family, at the junctions of the leading cells of the epidermis during dorsal closure. Steppke antagonizes further accumulation of myosin at the adherens junctions to relax cell–cell junctions, and precludes tissue disruption [77] (Figure 4B). Mechanical stretching of epithelial cells during wing imaginal disc growth in *Drosophila* results in a planar polarized distribution of myosin-II that

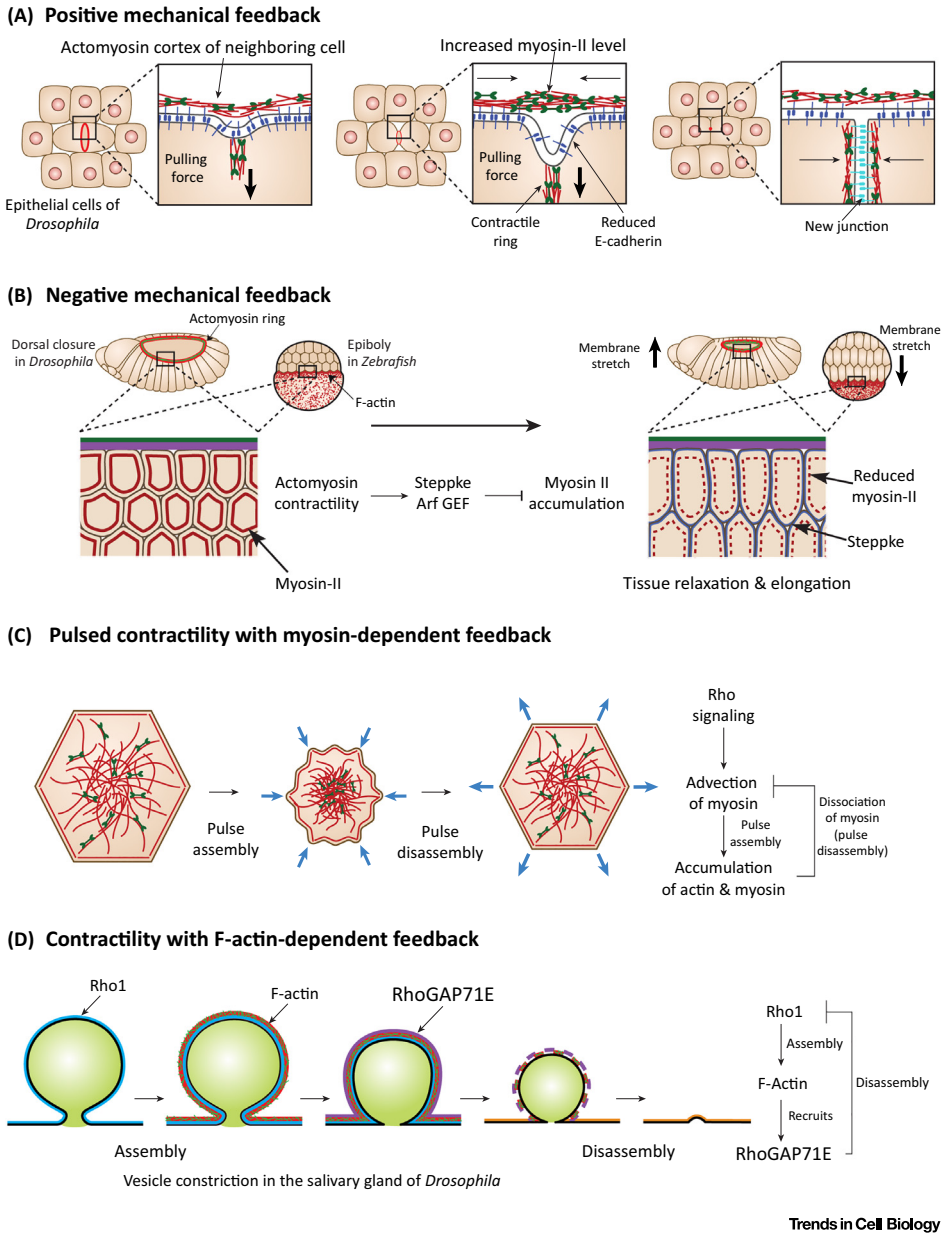

Figure 4. Mechanical Feedback Mechanisms Regulating Actomyosin Contractility. (A) Positive mechanical feedback. The pulling force (black arrow) generated by the ingressing actomyosin ring of a dividing cell leads to accumulation of myosin-II (green) in adjacent cells, and dilution of E-cadherin (navy blue) that promotes junctional remodeling and formation of new junctions (cyan) between the dividing and neighboring cells. (B) Negative mechanical feedback. Stretching of the epithelial tissue (black arrow) during dorsal closure in *Drosophila* and epiboly in *Zebrafish* recruits Steppke, an Arf-guanine nucleotide exchange factor (Arf-GEF) (blue), to the junctions, which negatively feedbacks on myosin accumulation (red) causing relaxation and elongation of the epithelial cells. (C) Pulsed contractility with myosin-dependent feedback. Contractile pulses of a medioapical actomyosin network during germ-band extension in *Drosophila* are regulated by myosin advection and dissociation. During pulse assembly, myosin advection recruits actomyosin network components at the cell center. Accumulated myosin-II negatively feedbacks to reduce advection, leading to pulse disassembly. (D) Contractility with F-actin-dependent feedback. Vesicle constriction in the salivary gland of *Drosophila* depends upon a cycle of F-actin assembly and disassembly. After fusion of the vesicle to the membrane, active Rho1 (blue) accumulates on the vesicles and triggers F-actin assembly (red). The assembled F-actin negatively feedback on Rho signaling via recruitment of RhoGAP71E (magenta), leading to F-actin disassembly.

orients cell division along the axis of stretch, resulting in a directional tissue elongation [78]. A mechanotransduction feedback loop in the *C. elegans* spermatheca is responsible for timely oocyte transit into the uterus after ovulation and fertilization. Mechanical stretch of the tubular tissue by the ovulating oocyte causes dissociation of the RhoGAP SPV-1 from the membrane which permits the increase in Rho activity that induces actomyosin contractility and embryo exit [79].

Besides tissue morphogenesis and homeostasis, mechanical tension plays a crucial role in tissue regeneration. Mechanically stretched leader cells of the epicardial tissue fail to divide and undergo endoreplication. The resulting large multinucleated leader cells have a higher surface covering capacity that helps in tissue regeneration [80]. Also, strain-based myosin recruitment, coupled with tension-based myosin stabilization at the wound edge, eventually leads to an increase in myosin levels around the wound necessary to overcome tissue resistance for wound closure in *Drosophila* [16].

Oscillation of actomyosin networks, a conserved phenomenon observed in multiple developmental processes, is caused by feedback loops of an activator-inhibitor system. A cycle of Rho activation via a RhoGEF, and Rho inhibition via a RhoGAP, maintains a self-organized system [36]. The self-organization also depends upon feedback regulation by downstream effectors of Rho signaling – myosin-II activity or F-actin. Pulsatile behavior of the actomyosin network during *Drosophila* germ band extension results from the positive and negative feedback by myosin advection and dissociation, respectively. Myosin-II induced flow facilitates positive feedback, amplifying the recruitment of actomyosin machinery and causing **pulse-assembly**. Once the concentration of myosin-II reaches its maximum, it negatively feedbacks myosin-II advection, resulting in disassembly of the pulse [81] (Figure 4C). Contrary to this, dynamic patterns of Rho signaling can be independent of myosin-II. RhoA pulses in the embryos of *C. elegans*, *Xenopus*, and starfish persist in the absence of myosin-II activity, and instead, depend upon F-actin mediated negative feedback regulation of Rho signaling [59,82]. While the identity of the Rho inhibitor is still unknown in the oocyte and embryos of *Xenopus* and starfish, RHO-1 pulses in *C. elegans* embryos are terminated by the F-actin mediated recruitment of two redundantly acting RhoGAPs (RGA-3/4) which provide negative feedback for Rho activation [59]. Contractile cycles of Rho1 observed during vesicle constriction in the salivary glands of *Drosophila* also depend upon negative feedback regulation by F-actin. F-actin recruits the RhoGAP, RhoGAP71E, leading to Rho1 inactivation [33] (Figure 4D).

Concluding Remarks and Future Perspectives

Significant progress in recent years has highlighted the role of contractility in multiple developmental processes at the cellular and tissue level. Here, we attempted to group many of those findings under unifying general principles of actomyosin regulation. Yet, our understanding of the mechanisms of regulation of contractility at the molecular level *in vivo* is still far from complete (see Outstanding Questions). Previously, we assembled a network of actomyosin regulators – the contractome – based on *in vitro* and cell culture systems [83]. A challenge for future studies is to ascertain which of these regulators are relevant in the multicellular context. In particular, a systems level approach to study the complete contractome in an entire animal at single cell resolution will be necessary to de-convolve the complexity of the network of actomyosin regulators. Such an approach is becoming feasible with genome editing to endogenous proteins, and advanced *in toto* imaging techniques for imaging the whole organism in its native state. Quantitative information provided by high-resolution imaging can be used to develop numerical models that will help in identifying different modes of

Outstanding Questions

Which components of the actomyosin machinery are used to build each of the actomyosin structures found *in vivo*, and what is their unique architecture?

What are the genetic and mechanical factors responsible for the spatial and temporal regulation of Rho GTPase signaling in different developmental contexts?

Are the same regulators (e.g., GEFs and GAPs) used for the same morphogenetic event (e.g., cytokinesis) throughout development and in adults, or do they change depending on tissue or timing?

Are there tissue specific functions for the same regulators, or is the function of a regulator constant between tissues? And how conserved are these functions between species?

How is it that cell adhesion structures positively regulate contractility in some contexts, and negatively regulate contractility in other contexts?

What are the mechanosensors and mechanotransduction feedback pathways activated in response to persistent, transient, or cyclic contractility *in vivo*?

actomyosin contractility regulation employed during complex morphogenetic events. While tools for genetic perturbation of various regulators are well established in most model organisms, mechanical perturbation and force measurements *in vivo* are still an open challenge. Laser ablation is a widely used invasive method for measuring relative tension, but the field is still missing easy to use tools for measuring and manipulating contractile forces *in vivo* with high precision. Interestingly, a recent study has reported an optogenetic tool for precise spatiotemporal activation of Rho signaling which drives apical constriction and tissue invagination in *Drosophila* embryo [84]. Several novel noninvasive techniques are being developed for applying as well as quantifying force [85,86]. Given these technological advancements, we expect to gain further insight into the fundamental principles of actomyosin regulation employed in living animals.

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