Architecture shapes contractility in actomyosin networks

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Abstract

Myosin-driven contraction of the actin cytoskeleton is at the base of cell and tissue morphogenesis. At the molecular level, myosin motors drive contraction by sliding actin filaments past one another using energy produced by ATP hydrolysis. How this microscopic sliding activity gives rise to cell-scale contractions has been an active research question first in muscle cells, and over the last few decades in non-muscle cells. While many early investigations focused on myosin motor activity, increasingly, the nanoscale architecture of the actin network emerges as a key regulator of contractility. Here we review theoretical and *in vitro* reconstitution studies that have uncovered some of the key mechanisms by which actin network organization controls contractile tension generation. We then discuss recent findings indicating that similar principles apply in cells.

Introduction:

Actomyosin contractility is at the base of cellular morphogenesis and mechanosensing. Shape changes of animal cells as they divide, migrate, or form tissues in developing embryos, are driven by gradients in actomyosin contractility (reviewed in [1,2]). Furthermore, contractile forces exerted on neighboring cells and the extracellular matrix allow cells to sense the stiffness of their environment, which influences cell migration, cell shape dynamics within tissues, as well as cellular fate decisions (reviewed in [3]).

The mechanisms of actomyosin contractility were first investigated in striated muscle cells [4]. In these cells, actomyosin is arranged in highly ordered, one-dimensional arrays called sarcomeres, where network architecture is perfectly adapted for contractility generation (Fig. 1a). In non-muscle cells, actomyosin structures are generally less ordered, ranging from stress fibers, where actin filaments are bundled but lack clear polarity, to the cellular cortex, where actin forms a mostly isotropic network (Fig. 1b). In such disordered structures, contractile tension generation cannot be understood in terms of a sarcomeric mechanism. Partly due to the difficulty in obtaining information on the spatial organization and dynamics of actin filaments in cellular networks, the mechanisms controlling actomyosin tension generation in non-muscle cells have long remained elusive. In fact, in morphogenesis studies, cortical contractile tension has often been assumed to be simply proportional to the levels of myosin II at the cortex [5-8], with little attention paid to the organization of the actin network itself.

Yet, *in vitro* studies of cell-free actomyosin systems reconstituted from purified components, as well as theoretical models clearly indicate that the spatial arrangement and physical properties of the actin filaments in the network are, alongside myosin motors, key to tension regulation (reviewed in [9]). Several recent studies in cells indeed indicate that changing actin network organization can strongly affect contractile tension even when myosin activity remains unchanged [10,11]. Increasingly, the nanoscale architecture of the actin network emerges as a key regulator of contractility both *in vitro* and *in vivo*.

We review here *in vitro* and theoretical studies demonstrating that different aspects of actin network architecture are key regulators of contractile tension generation. We also discuss recent findings indicating that similar principles apply in cells. Finally, we highlight some of the important open questions in the field.

I. Tension generation: why do contractile forces dominate?

Actomyosin contractility predominantly results from the mechanical action of myosin II motors, which use energy released from ATP hydrolysis to exert forces on actin. Actin filaments (F-actin) have structural polarity conferred by the head-to-tail assembly of the actin monomers, leading to two distinct filament ends denoted as the *minus* (or pointed) and *plus* (or barbed) ends. Myosin II motors have two globular head domains joined by a long tail domain. The head domains bind to actin filaments and selectively move towards the plus ends, while the tail domains serve to assemble myosin II molecules into bipolar filaments with motor heads on the two ends and tails packed in the center. This bipolar architecture allows myosin filaments to slide anti-parallel actin filaments with respect to each other. Depending on the arrangement of the actin filaments, the sliding activity can give rise to either a contractile or an extensile force (Fig. 2a). Yet, all known cellular actomyosin structures are overall contractile, indicating that some physical bias results in contractile forces dominating.

In skeletal muscle, this bias unequivocally originates from the arrangement of actin and myosin in repeating aligned arrays called sarcomeres, where myosin bipolar filaments are localized in between antiparallel actin filaments having their minus ends inwards and their plus ends outwards and anchored at Z-disc regions in between sarcomeres (Fig. 1a). The localization of the myosin clusters in the vicinity of F-actin minus ends and the co-alignment of myosin and actin filaments convert the sliding activity of the motors into uniform contraction [12]. Some actin structures in non-muscle cells such as stress fibers and contractile structures associated with integrin-based adhesions display some level of sarcomeric order, but with varied polarity patterns [13,14]. In strong contrast, the actomyosin cortex lacks any apparent order, making contractile and extensile actomyosin configurations equally likely (Fig. 2a) [15]. The search for mechanisms that bias isotropic actomyosin networks towards

contraction has been an active research focus for both experimentalists and theorists for the last two decades.

Broadly speaking, the mechanisms proposed so far ascribe the bias towards contractility either to mechanical asymmetries or to self-organization of contractile force dipoles (Fig. 2 b,c). Most mechanical models attribute contractility to the nonlinear elastic properties of actin filaments. Since actin filaments are semi-flexible with a persistence length around 10 μm [16], they strongly resist tension but filament portions longer than ~ 0.3 μm readily buckle under compressive forces comparable to those generated by single molecular motors (~ 2 pN). Buckling relaxes extensile configurations, and can thus bias actomyosin bundles and networks towards contraction (Fig. 2b) [17,18]. An important prerequisite is that forces can propagate across the system, meaning that the actin filaments need to be crosslinked. Strong experimental support for this mechanism comes from direct observations of buckling of individual fluorescently labeled actin filaments during contraction of disordered bundles and 2D networks *in vitro* [17,19,20]. The degree of shortening of filaments by buckling correlates with the macroscopic shrinkage of the networks, further supporting the buckling model [19,21]. Buckling can sometimes lead to filament severing, which further promotes contraction [19].

Experimental evidence of a buckling mechanism promoting contractility in cells is at this point missing. A potentially crucial difference between cellular networks like the cortex and *in vitro* biomimetic systems is the length of the actin filaments between crosslinking points. In biomimetic assays filaments tend to be longer than the persistence length and the distance between adjacent crosslinks is on the order of 1 μm [22]. In contrast, recent work suggests that the cortex is made of filaments much shorter than the persistence length and contains a mixture of formin-nucleated filaments with lengths on the order of 1 μm, and much shorter Arp2/3-nucleated filaments in the 100 nm range [23]. Furthermore, typical cortical mesh sizes range between 30 and 200 nm, suggesting very short distances between crosslinkers [24,25]. Actin filament segments between crosslinking points may therefore be too short and rigid for buckling to strongly contribute to contractility generation in cellular networks.

Self-organized polarity has been proposed as an alternative contractility-promoting mechanism. The best-studied example is motor-driven polarity sorting of actin filaments. The basic idea is that if motors processively walk along actin filaments and slow down or stall before they detach from the plus end, they can cause polarity sorting of the filaments into radial arrays referred to as asters, connected by cables of antiparallel actin filaments that resemble sarcomere-like contractile structures [26] (Fig. 2c). Theoretically, polarity sorting could also be achieved via actin treadmilling: if actin filament plus end elongation and minus end shrinkage are faster than myosin movement, treadmilling can bias myosin to F-actin minus ends, which is a contractile configuration similar to that in sarcomeres [27] (Fig. 2c).

In networks, a clear signature of polarity sorting should be the formation of radial arrays of filaments (asters), where the filament ends point inwards and motors accumulate in the center. *In vitro*, such aster-like arrangements have been observed both in microtubule-motor mixtures [28] and in actomyosin networks [29-31]. In many cell types, the cortex also tends to form aster-like actin structures and compact myosin foci. However, it is unclear to what extent these are due to polarity sorting, or to other patterning processes driven by actin nucleation or RhoA-mediated regulation of myosin [32-34].

In addition to these mechanisms, simulations suggest that the finite size of myosin bipolar filaments may promote contraction by favoring rotation of myosin filaments toward low-energy contractile configurations [35,36] or by generating anisotropic forces by myosin heads at the filament ends [37]. To our knowledge, these predictions have not been tested experimentally.

II. Network architecture and tension: the importance of being well connected

Though it remains unclear which of the mechanisms described above determines the bias towards contractility in cellular networks, network connectivity via actin crosslinkers is a key determinant in all current models (Fig. 2). Interestingly, recent work highlights that further to promoting contractility, crosslinking also tunes the length scale of contractions and the magnitude of the stresses developed.

Cell-free experiments demonstrate that long-range coordinated contraction only occurs above a threshold connectivity provided by crosslinks [38]. This threshold was identified as the percolation threshold, where all filaments are connected so that motor-driven stresses propagate across the entire system [38,39]. Below the percolation threshold, myosins contract the network only on short length scales, creating small clusters [31,38]. In cellular actomyosin networks, the role of crosslinking in actomyosin tension generation is harder to investigate because of redundancies between the vast array of different crosslinkers present. Nonetheless, several recent studies have highlighted the importance of crosslinking for cellular contractility. Experiments in *C. Elegans* embryos showed that crosslinking by plastin is required for effective long-range cortex contraction [11]. Similarly, depletion of the actin bundling protein Eps8 decreases cortical tension in cancer cells [40], and depletion of different cross-linkers, including alpha-actinin and fascin, decreases cellular rounding force, a readout of tension, in mitotic cells [41].

Interestingly, excessive crosslinking can be counter-productive and limit contractile tension generation. Early experiments investigating bulk contractility in *in vitro* actomyosin networks have already shown that a minimal level of cross-linking is required for contraction, but that excessive cross-linking is detrimental [42]. More recent theoretical and *in vitro* work has further dissected the relationship between crosslinking, motor activity and network contractility [39,43,44]. The emerging view is that maximal contractile tension is achieved at an intermediate level for crosslinking. Consistent with this idea, a recent study combining experiments on the cellular cortex and simulations suggests that cortical tension is maximum at intermediate actin filament lengths [10]. This tension optimum likely results from intermediate connectivity favoring contractility build up, as at a given crosslinker density, filament length directly correlates with network connectivity.

How exactly excessive connectivity limits contractility is not well understood. One possible explanation is that at intermediate connectivity, the network can remodel in response to myosin-mediated stresses, and that such remodeling favors contractile configurations [10]. In contrast, remodeling is limited in an overly connected network, leading to lower tension. Network remodeling could promote contractile tension by mechanisms like polarity sorting (Fig. 2c), or by the relaxation of extensile

stresses by a buckling-like behavior of local network configurations. While these are plausible mechanisms, they remain speculative largely due to the difficulty in obtaining microscopic data on the dynamics of single actin and myosin filaments in dense contractile networks, particularly in cells.

In cells, connectivity is modulated by network turnover. Crosslinkers and actin filaments turn over within seconds and tens of seconds respectively [1]. Microscopic simulations indicate that maximum tension is expected for an optimum ratio of the filament and cross-linker turnover rates [36]. Theory and experiments further suggest that turnover could deeply affect network behavior. Turnover is thought to stabilize dynamic contractile steady states with a homogeneous myosin distribution, as opposed to irreversible contraction into clusters displayed by networks that lack turnover [43,45-47]. Interestingly, a number of models indicate that turnover should promote cyclic contractions, where the actomyosin network continuously pulses [44,48,49]. Such pulsed contractions appear to be very common in cell and tissue morphogenesis (reviewed in [2]).

Mechanosensitive modulation of bond lifetimes could also affect network connectivity and tension. For instance, in *in vitro* actin networks crosslinked with fascin, myosin was shown to cause crosslink unbinding at high motor densities [38]. Rather than being prevented from contracting at high crosslinking levels, the network was actively ruptured into clusters. Intriguingly, while fascin appears to form slip bonds, whose lifetime decreases with loading so fascin is depleted from regions of high contractile stress [38], other cross-linkers, like α-actinin-4, form catch bonds and accumulate in stressed regions [50]. Mechanical stress has also been shown to affect the rates of formin-based actin filament elongation, with load making elongation faster for the mammalian Dia1 and budding yeast Bni1 formins [51,52], and slower for the fission yeast formin Cdc12 [53]. Myosin II motors themselves also form catch bonds [54]. How mechanosensitive effects influence tension generation has not been systematically investigated.

Finally, in addition to the level of connectivity, the specific geometric arrangement of actin networks could also influence tension. An interesting recent *in vitro* study used

surface micropatterning to impose actin filament arrangement into either bundled or branched structures with disordered (mixed polarity) or ordered (antiparallel) filaments [39]. Upon exposure to myosin motors, Arp2/3-mediated branching appeared to promote contractility the most, possibly by providing more efficient percolation than bundling proteins. In contrast, several studies suggest that in cells, Arp2/3 acts to reduce contractile tension at the cortex [55]. Decreasing Arp2/3 activity promotes the formation of cellular blebs, protrusions that grow because of cortex contractions [55-57], and enhances myosin II-powered retrograde flow in neuronal growth cones [58]. These observations suggest that Arp2/3-nucleated actin networks may not provide an optimal scaffold for tension generation at the cell cortex. However, the microscopic basis of Arp2/3 effect on tension remains unclear, as information on cortical actin filament arrangement is still very limited (reviewed in (Chugh and Paluch, submitted)).

Conclusions and open questions

The last decade has seen considerable progress in our understanding of contractility in non-sarcomeric actomyosin networks. *In vitro* assays and modeling have been particularly crucial in identifying the basic mechanisms of tension generation in a disordered network. Recent studies *in vitro* but also in cells have clearly shown that the nanoscale architecture of the actin network is a crucial determinant of tension, and that the level of crosslinking is a particularly important parameter. While we focused here on the actin cortex, cell contractility mediated by stress fibers also appears to depend on the architecture and connectivity of the fiber network [59],. Recent work also suggests that at a supracellular level, actomyosin networks architecture can be tuned in response to mechanical constraints to control force orientation during tissue morphogenesis [60].

Despite an increasingly refined understanding of contractility *in vitro* and despite detailed knowledge of the molecular composition of the actomyosin 'contractome' [61], the physical mechanisms controlling contractility in cells, particularly at the actomyosin cortex, remain insufficiently understood. This is partly due to inherent limitations of *in vitro* studies and modeling, where network components are usually restricted to actin, myosin and crosslinkers. Such reductionist approaches are powerful, as network composition is fully tractable, making predictions quantitative and precisely testable. However, such approaches can of course only unveil mechanisms relying on the specific components investigated. Cellular networks typically contain >100 regulatory proteins [62], many of which directly or indirectly affect tension [41].

Another key challenge is the difficulty in uncovering the organization and dynamics of actin filaments and actin-binding proteins in dense actomyosin networks. Super resolution microscopy and improvements in electron microscopy are starting to overcome this limitation. Recent studies have for example successfully used advanced image analysis and super-resolution microscopy to beat the diffraction limit and investigate the thickness and organization of the cellular cortex [10,63], or to follow myosin minifilaments during cortex contractions [64,65]. Dissecting the nanoscale

architecture of cellular actin networks and investigating how they dynamically remodel as cells contract and deform constitutes an exciting future research avenue.

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Figure legends:

Figure 1: Actomyosin networks in cells.

a) In muscle cells, actin and myosin are organized into sarcomeres. The ordered organization of a sarcomere promotes contractility. Top: image of sarcomeres in *Drosophila* flight muscle; red: actin (phalloidin), green: the myosin-rod-associated protein flightin-GFP, image obtained with permission from [66]. Bottom: schematic of the organization of a sarcomere. **b)** In non-muscle cells actomyosin networks are less ordered. Cell images, kindly provided by Murielle Serres (Paluch lab), represent HeLa cells in interphase and mitosis, where DNA (red) and F-actin (cyan) have been labeled. Scale bars: 10 μm.

Figure 2: Tension generation in isotropic actomyosin networks.

a) In a disordered network, contractile and extensile actomyosin configurations are equally likely. **b)** Filament buckling can relax extensile forces and bias the network force distribution towards contraction**. c)** Under certain kinetic conditions, myosin minifilament processive walking or actin turnover could bias networks towards contractile configurations (details in text).

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This paper uses super-resolution microscopy to investigate the density distribution of the actin cortex. It shows that actin density is not homogenous through the cortical layer, and that a gap of 10-20 nm exists between the cortex and the plasma membrane; the size of this gap could affect the interactions between the cortex and the membrane. This paper presents one of the first insights into the nanoscale architecture of the actin cortex in live cells.

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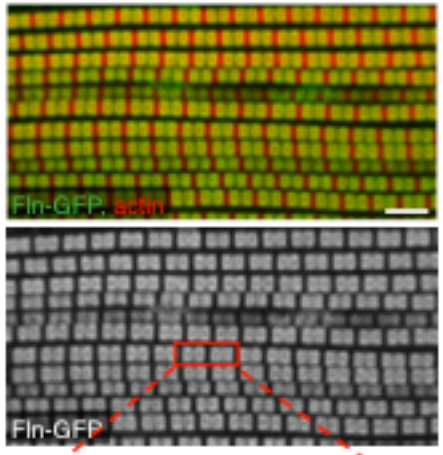
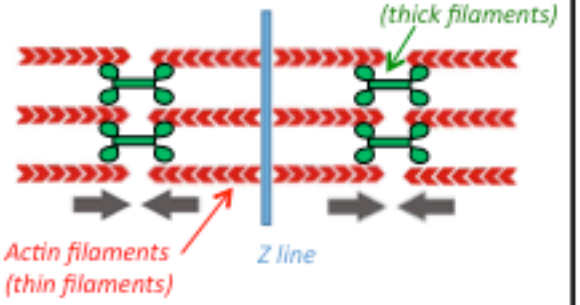
a) Muscle cells

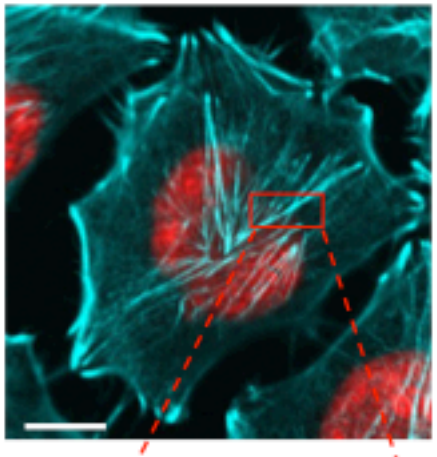




Sarcomere

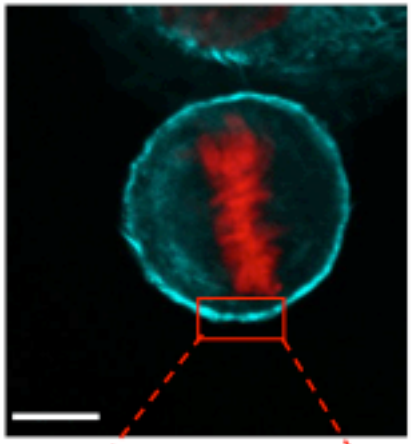
b) Non-muscle cells

Interphase cell

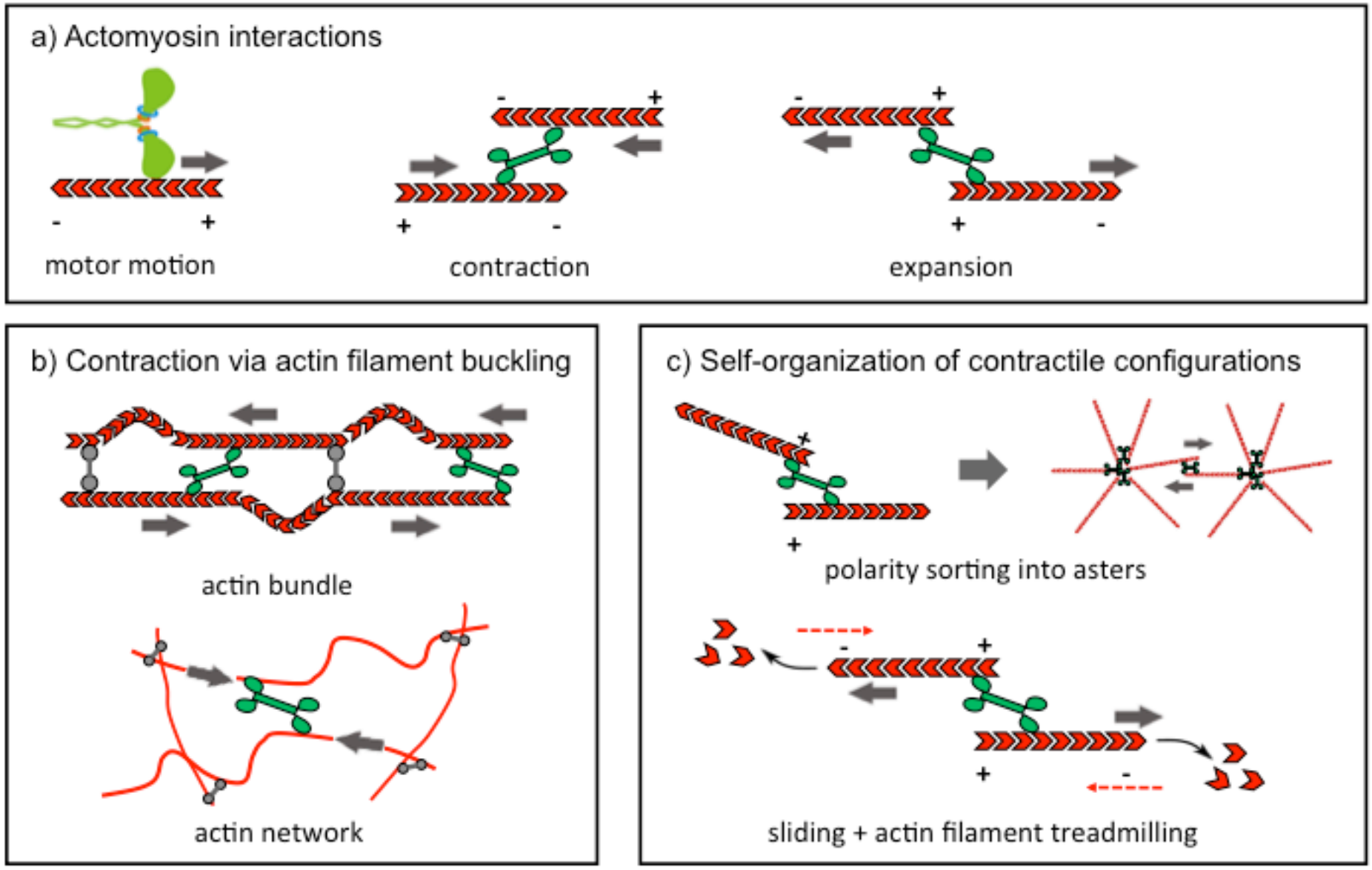


Stress fiber

Mitotic cell



Actomyosin cortex


a) Actomyosin interactions
motor motion
contraction
expansion
b) Contraction via actin filament buckling
actin bundle
actin network
c) Self-organization of contractile configurations
polarity sorting into asters
sliding + actin filament treadmilling