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REVIEW

SUBJECT COLLECTION: MECHANOTRANSDUCTION

How the mechanobiome drives cell behavior, viewed through the lens of control theory

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ABSTRACT

Cells have evolved sophisticated systems that integrate internal and external inputs to coordinate cell shape changes during processes, such as development, cell identity determination, and cell and tissue homeostasis. Cellular shape-change events are driven by the mechanobiome, the network of macromolecules that allows cells to generate, sense and respond to externally imposed and internally generated forces. Together, these components build the cellular contractility network, which is governed by a control system. Proteins, such as non-muscle myosin II, function as both sensors and actuators, which then link to scaffolding proteins, transcription factors and metabolic proteins to create feedback loops that generate the foundational mechanical properties of the cell and modulate cellular behaviors. In this Review, we highlight proteins that establish and maintain the setpoint, or baseline, for the control system and explore the feedback loops that integrate different cellular processes with cell mechanics. Uncovering the genetic, biophysical and biochemical interactions between these molecular components allows us to apply concepts from control theory to provide a systems-level understanding of cellular processes. Importantly, the actomyosin network has emerged as more than simply a 'downstream' effector of linear signaling pathways. Instead, it is also a significant driver of cellular processes traditionally considered to be 'upstream'.

KEY WORDS: Myosin II, Control system, Setpoint control, Feedback

Introduction

Control over cell shape changes is vital for processes ranging from cytokinesis and cell migration to more complex events, such as tissue development and homeostasis, wound healing and immune function. Cells have evolved to respond to mechanical stimuli, including stretch, compression and shear forces, as well as internally generated tension and strain. We define the mechanobiome as the set of macromolecules that allows cells to generate, sense and respond to such forces (Surcel et al., 2019; Kothari et al., 2019). The mechanobiome incorporates many components of the cytoskeleton, including myosin II motor proteins, actin crosslinkers, scaffolding proteins, actin polymerization and depolymerization factors, small GTPases and other cytoskeletal polymer systems (Wu et al., 2008; Robinson et al., 2012; Blanchoin et al., 2014; Zaidel-Bar et al., 2015; Chugh and Paluch, 2018; Dogterom and Koenderink, 2019). This term includes components that build cell–cell and cell–matrix interactions ('adhesome'), as well as all the proteins and networks commonly included in the 'contractome' (Zaidel-Bar et al., 2015; Horton et al., 2016). The benefit of the larger umbrella term 'mechanobiome' is that it recognizes that each of these seemingly discrete systems are integrated with one another. In addition, although actin, microtubule and intermediate filament networks have often been considered independently, these networks really work synergistically to drive cellular processes (Zhou et al., 2010; Huber et al., 2015; Henty-Ridilla et al., 2016; Dogterom and Koenderink, 2019).

The cell cortex is an ∼200–500-nm-thick composite material that includes the plasma membrane and the dense actin meshwork that lies just below the membrane (Fig. 1) (Reichl et al., 2008; Robinson et al., 2012; Clark et al., 2013). Along with the underlying and penetrating cytoplasm, the cell cortex contributes to the mechanical properties of a cell (see Glossary). Cells are active viscoelastic fluids, and the collection of mechanical stresses at the cell cortex leads to a cortical (surface) tension that serves to minimize the ratio between surface area and volume of the cell, in essence a rounding pressure (Robinson et al., 2012; Moeendarbary and Harris, 2014). The membrane itself constitutes an upper limit of ∼2–5% of the total cortical tension value (Luo et al., 2014). The remaining active and passive portions of cortical tension are largely generated by the cytoskeletal network. These networks are viscoelastic in nature, allowing for time-scale-dependent behaviors in response to mechanical stresses (Gardel et al., 2004; Robinson et al., 2012; Pegoraro et al., 2017; Chugh and Paluch, 2018). The elastic component is derived from the strong interactions between actin filaments and crosslinkers that build the cytoskeletal meshwork, whereas the viscous component is a result of those structures remodeling over time to relieve the stress imposed on the system (see Glossary).

In this Review, we focus on the biochemical interactions and feedback mechanisms that regulate the cytoskeletal network primarily at the cellular level. Specifically, we apply theoretical principles from control engineering to cellular systems to understand how the actomyosin network influences cell behaviors (Box 1). Cells integrate biochemical and mechanical inputs through the use of control systems that rely on sensors, actuators and feedback loops. By providing examples of control systems in the context of the actin cytoskeletal network, we review mechanisms that regulate the setpoint (baseline) through differential protein-binding affinities, post-translational modifications, force-sharing across a network and complex assemblies. Additionally, we explore how seemingly unrelated processes (e.g. gene regulation, metabolism and cell fate determination) and cell mechanics feedback onto each other to orchestrate complex cellular events. We primarily use myosin II as an example of a protein that functions both as a sensor and actuator in the context of setpoint and feedback. However, these concepts also apply more broadly to many other components of the mechanobiome. We aim to demonstrate that the application of control theory principles will provide systems-level insight into how cells respond to the forces

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Glossary

Control system: a system that integrates inputs with outputs to determine the behavior of the system. See Box 1.

Cortical tension: energy cost for adding a unit of area to the surface of a cell. The cortical tension is derived from active and passive forces and material properties of the cortex.

Fluidization: as a result of motor and polymer dynamics, the cytoskeleton of the cell can have flow (liquid-like) characteristics. These flow properties help increase the remodeling of the network, effectively 'stirring the pot'. The network flow arises from polymer turnover and the motors 'kicking on the network' and helping crosslinkers and polymers to dissociate and disentangle. The flow helps move the system out of local energy minima, overcoming the soft-glassy characteristics often observed on shorter timescales for cytoskeletal systems. The fluidization results from the super-diffusive properties of the system.

Laplace pressure: pressure differential at a curved surface that minimizes the surface area to volume ratio.

Mechanoresponse: defined as the accumulation of a protein in response to an applied mechanical stress (internally generated or externally imposed).

Setpoint control: reference point, or baseline, at which the control system maintains the signal and/or response.

Strain stiffening: property of a material where an increase in deformation leads to an increase in stiffness.

Super-diffusive behaviors: properties of the cortex and cytoplasm of a cell where particle motion exceeds what is expected if the motion was purely driven by thermal energy. Molecular motors and polymer dynamics are two major contributors to the super-diffusive behavior of the structure of a cell.

Viscoelasticity: the property of a material with viscous and elastic characteristics that allows for differential timescale-dependent behaviors. Elastic elements store energy independently of time, while viscous elements dissipate energy over time. Cells generally have viscoelastic characteristics, combining these features such that they store energy over short timescales, but then dissipate this energy over time as the cytoskeletal network, composed of polymers and noncovalently attached crosslinkers, remodels.

they encounter during normal and disease-state processes. It is worth noting that the mechanics of cell–cell junctions, cell–extracellular matrix and tissues have been recently reviewed elsewhere (Charras and Yap, 2018; Chen et al., 2018; Yap et al., 2018; Sun et al., 2019).

Control systems

To ensure robust cellular mechanical behaviors, the cytoskeletal network functions as a control system (see Box 1), which includes sensors to detect environmental cues or deviations away from a desired behavior, and actuators, which execute the desired behavior or process (Fig. 2A) (Ren et al., 2009; Kee et al., 2012; Srivastava and Robinson, 2015; Srivastava et al., 2016; Schiffhauer and Robinson, 2017; Schiffhauer et al., 2019). Inherently, control systems may be open- or close-looped. In the open-loop scenario, the actuator response is based on sensed changes to an input. In the closed-loop situation, the system monitors the progress of the desired behavior or process and makes adjustments, thus closing positive- and/or negative-feedback loops. The system behavior is measured against an adjustable setpoint, which establishes the reference point that the system uses to define baseline (Fig. 2B). By regulating the setpoint, each individual system is poised to respond differently to disturbances. All components, sensors, actuators, feedbacks and setpoints are tunable.

In the cell, macromolecules, such as receptor proteins, small GTPases, metabolites and mechanoresponsive proteins (proteins that sense and accumulate in response to a local mechanical stress), function as sensors, receiving biochemical and mechanical inputs, and signaling to actuators. In many cases, proteins in a control system function as both sensors and actuators, allowing the cell to use the same system that generates the foundational mechanical properties to also adapt to constantly changing inputs. For instance, the force-sensing, actin-binding cooperativity and corresponding allostery in the actin filament allow myosin II to sense and respond to load (force) in the actin network, thus functioning as both a sensor and actuator (Galkin et al., 2012; Kee et al., 2012; Luo et al., 2012). Mechanoresponsive actin crosslinkers, such as α-actinin and filamin, also provide a similar functionality (Luo et al., 2013; Schiffhauer et al., 2016; Schiffhauer and Robinson, 2017).

One example for the action of a control system may be observed during cleavage furrow ingression, which is a critical step in ensuring genomic fidelity and therefore needs to be an incredibly robust process with the ability to adapt to a dynamic environment. Thus, relying on a control system that integrates many inputs and implements feedback would be advantageous. In fact, in *Dictyostelium*, cleavage furrow assembly is driven by the cellular contractility control system, which can be described in a few components: the cytokinetic signaling module (signal input), the plant, which includes the contractile machinery (myosin II and actin crosslinker cortexillin I, which are the sensors and actuators), and feedback loops constructed in part by IQGAP regulatory proteins (Fig. 2A,C) (Kee et al., 2012; Srivastava and Robinson, 2015). The cell must transition from its default setting of resisting shape change to coordinating a major shape-change event, thus requiring differential sensitivities, or thresholds, to mechanical stresses. First, the system has a setpoint that is tuned and varies across the cell cycle. Second, through feedback in response to internally or externally generated stress, the system tunes the amount of contractile proteins that accumulates at the cleavage furrow cortex, ensuring robustness.

Initially, the cytokinetic signaling module built from a kinesin 6 protein (Kif12 in *Dictyostelium*) and inner centromere protein (INCENP) provides the initial input that leads to recruitment of contractile proteins. The associated shift in setpoint is demonstrated by the changes in the amount of force that is required to elicit a myosin II mechanoresponse over the cell cycle. During anaphase, the system becomes extremely sensitive to mechanical stress, and low forces are sufficient to induce a myosin II mechanoresponse (Effler et al., 2006; Ren et al., 2009). However, the same magnitude of response requires a significantly higher force (~2.5-fold) during interphase and the early phases of mitosis. In fact, a metaphase cell can be clamped with a micropipette used to apply a defined amount of mechanical stress, and the myosin II will not undergo mechanoresponsive accumulation (mechanoaccumulation) until the cell enters anaphase. This setpoint appears to be established by the RacE small GTPase, as null mutants of *racE* require only low mechanical stresses throughout the cell cycle to trigger myosin II mechanoaccumulation. As RacE maintains certain actin crosslinkers (dynacortin), as well as regulators of myosin II (14-3-3 proteins), on the cortex, this mutant identifies two mechanisms for modulating setpoint control: force sharing and myosin II assembly regulation (see below) (Robinson and Spudich, 2000; Zhou et al., 2010; West-Foyle et al., 2018).

Furthermore, the above system tunes the amounts of contractile proteins that accumulate at a site of stress through a mechanical feedback loop (Kee et al., 2012; Srivastava and Robinson, 2015). IQGAP scaffolding proteins sense inputs from small GTPases and also feedback onto the cytokinetic signaling module (Faix et al., 2001; Kee et al., 2012). Importantly, the cell has two IQGAP proteins that have opposing roles in modulating mechanoaccumulation: IQGAP1

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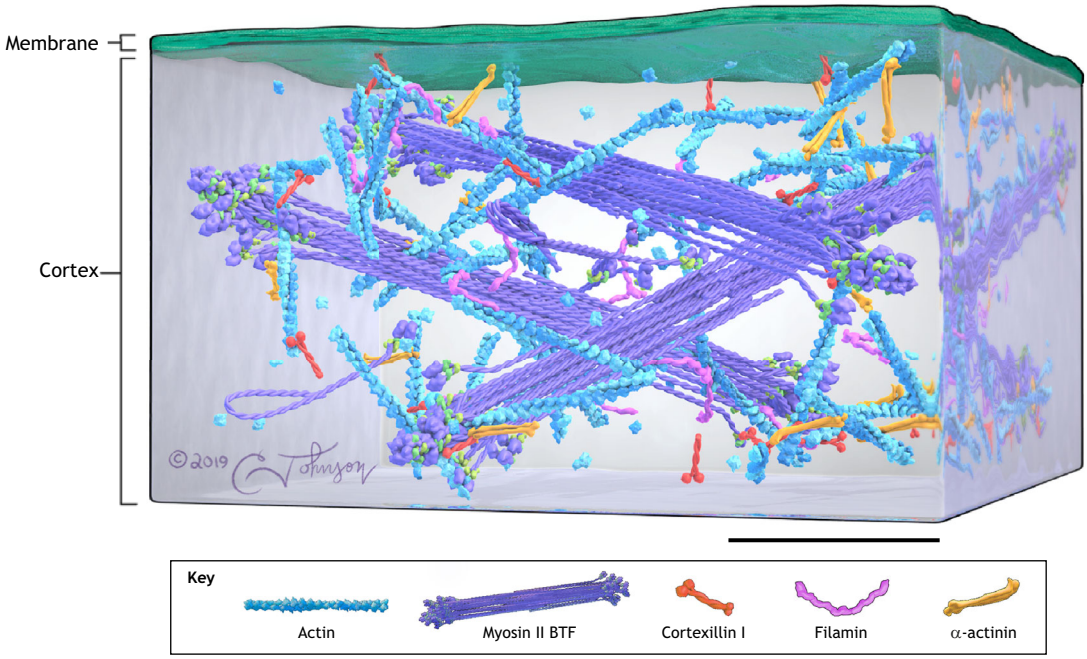

Fig. 1. Example components of the mechanobiome in *Dictyostelium*. Illustration of the cortical actin network that includes various cytoskeletal components. Proteins are modeled after existing PDB structures and reflect approximate concentrations, numbers, and lengths of crosslinkers and filaments from *Dictyostelium* (Condeelis et al., 1984; Goldmann and Isenberg, 1993; Faix et al., 1996; Mahajan and Pardee, 1996; Reichl et al., 2008; Robinson et al., 2012; Luo et al., 2013; Kothari et al., 2019). Scale bar: 100 nm.

provides an inhibitory function while IQGAP2 alleviates this inhibition. IQGAP1 binds cortexillin I with a 2:1 stoichiometry, while IQGAP2 only interacts at a 1:1 stoichiometry with cortexillin I. Thus, IQGAP1 may sequester cortexillin I, and likely myosin II, preventing their mechanoresponsiveness, and IQGAP2 then competes off IQGAP1, freeing cortexillin I and myosin II (Kee et al., 2012; Srivastava and Robinson, 2015; Kothari et al., 2019). This cross-antagonism and feedback between the signaling module, the IQGAP proteins and the contractile machinery regulates the setpoint of the control system. This control system allows for a 3–5-fold dynamic range in myosin II accumulation. The same system applies to interphase cells, but only a 2–3-fold dynamic range in myosin II mechanoaccumulation is observed, presumably because the loop extending to the mitotic spindle proteins is absent (Kee et al., 2012).

Using theoretical concepts from control engineering, we could explain the biphasic nature of the mechanosensory response of cells (Luo et al., 2013; Mohan et al., 2015). Myosin II accumulation is biphasic, first accumulating to resist deformation, then contracting against load and finally dissipating as the deformation is corrected. Previously, mathematical models had been used to explain the experimentally observed accumulation of contractile proteins at the site of mechanical perturbations (Luo et al., 2012). However, these models could not explain the biphasic nature of these accumulations. Incorporating the role of myosin II as a sensor and an actuator that provides force feedback was an important step in explaining the system.

The architecture of control systems is also observable in other processes involved in cell motility and tissue morphogenesis, including those driven by excitable, pattern-forming and self-organizing systems. In *Xenopus laevis*, waves of contractility regulators lead to cortical remodeling that eventually coordinates furrow formation (Bement et al., 2015). In *Dictyostelium*, the signal transduction excitable network (STEN) promotes protrusions and cytoskeletal reorganization that lead to cell migration. Decreasing the threshold, or setpoint, of STEN by synthetically decreasing the levels of phosphatidylinositol (4,5)-bisphosphate alters the actin-based protrusive activity and drives transitions between distinct migratory modes (Miao et al., 2017). Similarly, the positive and negative feedback through phosphorylation and dephosphorylation cycles of the myosin light chain kinase and the resulting contractions of myosin II generate a self-organized control system that regulates the pulsatile contractions that promote tissue morphogenesis during germ band extension in *Drosophila melanogaster* (Munjal et al., 2015).

Box 1. Control systems

Control engineering aims to understand the means by which systems ensure desirable, robust behavior in the presence of disturbances or perturbations. In engineered systems, a controller is used to regulate the desired behavior. The control process typically involves feedback and feedforward loops which are implemented using the following components.

Controller: controllers use the sensed signal and determine the corrective action, if any, that is required.

Sensor: sensors are used to discern the state of the system.

Actuator: actuators implement the desired action on the process, so as to achieve the requisite behavior.

Plant: the plant includes the sensors and actuators.

For example, a cruise control system ensures that the velocity of a car remains at the desired level – referred to in control systems as a setpoint – regardless of external disturbances such as changes in the slope of the road or wind gusts that would otherwise alter the speed. In this case, the sensor is the speedometer, and the actuator is the connection to the accelerator. The control system compares the desired and actual velocity and uses this information to determine whether the accelerator or brake should be depressed and by how much. The use of control engineering as a means of understanding biological systems has a long history, dating from Norbert Weiner's work on cybernetics. More recently, control theory has been used to understand various biological processes (see Iglesias and Ingalls, 2010 and the references therein).

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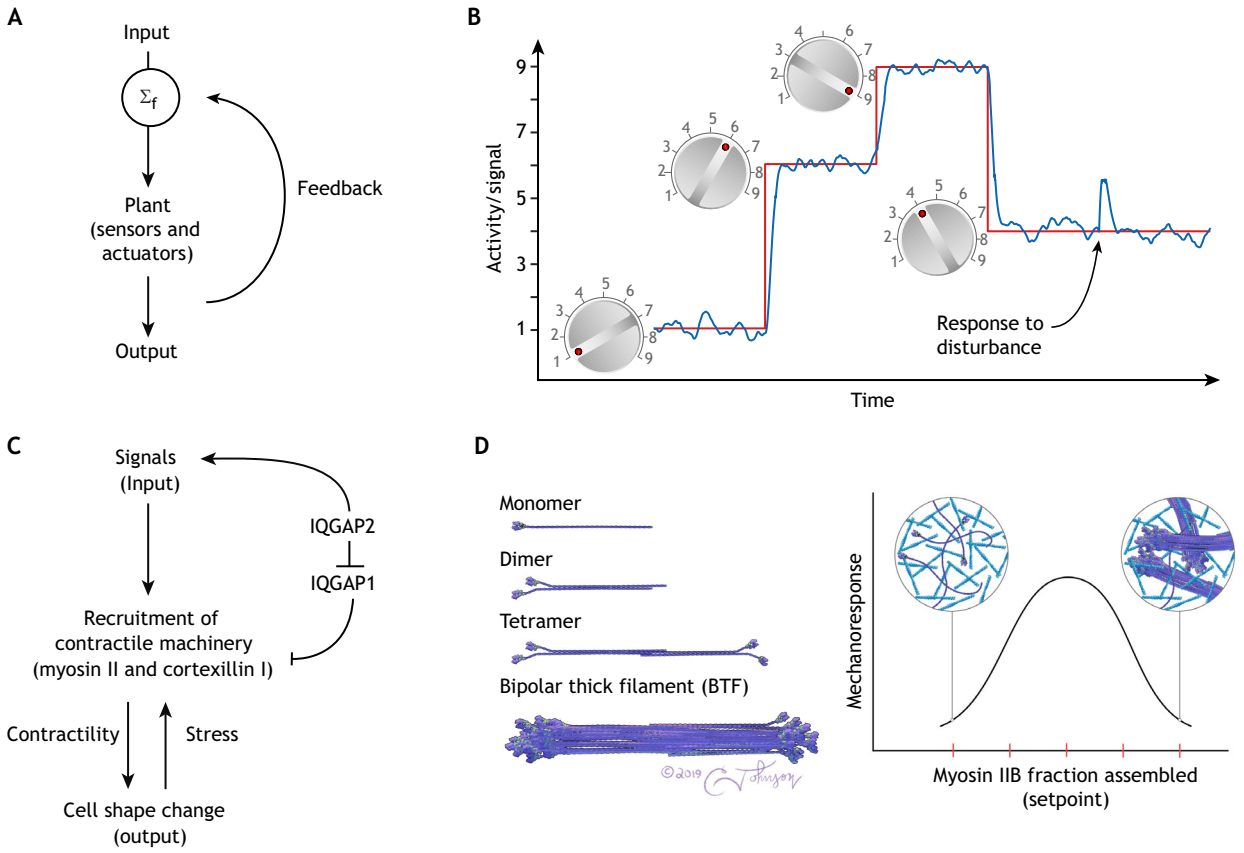

Fig. 2. Control systems and setpoint theory. (A) In a control system, the plant (sensors and actuators) translates input signals into an output behavior. The resulting changes can alter the input sum of forces (Σ_f), which then continues to influence the plant. (B) The setpoint defines the positioning of the system and magnitude of the response to a disturbance. Each horizontal red line and dial indicates a setpoint, the baseline response that the system maintains. The blue line indicates the measured activity or signal, for example myosin II accumulation at the cleavage furrow cortex. A disturbance can result in a response, and the system functions to restore the baseline after the response. The setpoint can shift over time (vertical red line) owing to many factors, including changes in extracellular matrix, intracellular signals and shifts in the metabolic landscape. (C) In the cell, input signals, such as from signaling (e.g. those from kinesin 6 and INCENP during cell division) or from mechanical stresses, are sensed by the plant (contractile machinery). The plant generates the output behavior (cell-shape change) and feedback loops tune the behavior. In this system, myosin II and cortexillin I (the contractile machinery) serve as both sensors and actuators. Feedback is mediated by IQGAP2, which antagonizes the binding between IQGAP1 and the contractile machinery and allows for the formation of mechanoresponsive contractility kits (Kothari et al., 2019). (D) Schematic illustration of myosin II assembly from a monomer to a bipolar thick filament (BTF). The fraction of myosin IIB assembly establishes the setpoint for myosin IIB mechanoresponsiveness. Moreover, this setpoint has an optimum whereby the mechanoresponsiveness has a biphasic relationship where too little or too much assembly leads to little mechanoresponse with an optimum in between. The setpoint dial (red ticks) may be shifted by altering the assembly fraction (depicted in the insets with actin filaments in blue) to elicit a change in the mechanoresponse.

Establishing the setpoint

By shifting the baseline, or setpoint, of a control system, a single cell can have different sensitivities and outputs in response to biochemical and mechanical cues (Fig. 2B). The setpoint may be established by varying protein affinities, post-translational modification states, the assembly state of the protein(s) and protein–protein interactions. For example, an optimal actin-binding affinity is required for actin crosslinkers filamin and α-actinin to be mechanoresponsive. If the actin-binding affinity is too low, the crosslinker is prevented from binding the network sufficiently, whereas in the case of a very high binding affinity, sufficient turnover to allow the dynamic behavior of the protein is inhibited (Schiffhauer et al., 2016). Modeling this relationship between actin-binding affinities and mechanoresponse allowed for the accurate prediction of which mammalian crosslinker isoforms would mechanorespond. Specifically, theory predicted and experiment verified that filamin B and α-actinin 4 mechanorespond strongly. In contrast, theory predicted that α-actinin 1 would not respond and filamin A would do so very weakly and over a much longer timeframe; neither protein displayed mechanoaccumulation experimentally (Schiffhauer et al., 2016). These observations suggest that the actin-binding affinity at the level of the interaction between a single actin-binding domain and actin helps define the mechanoresponsive ability and the kinetics of the response. By changing the expression patterns of these isoforms, modulating effective affinities through post-translational modifications or through steric hindrance caused by protein–protein interactions, cells may alter their setpoints and thus their mechanoresponsive abilities.

Setpoint control also provides insight into the functions and behaviors of different cell types. The baseline fraction of assembly of non-muscle myosin IIB into bipolar thick filaments (BTFs) predicts its mechanoresponsive ability across a wide range of cell types, including NIH 3T3 fibroblasts, HeLa and Jurkat cells (Schiffhauer et al., 2019). The mechanism underlying this setpoint control depends upon phosphorylation of the myosin heavy chain, which serves to maintain the particular ratio of unassembled (free) to assembled (bipolar filaments) myosin IIB subunits for each cell type. In Jurkat cells, 20% of myosin IIB is assembled and robustly mechanoresponds. 3T3 and HeLa cells, in which myosin assembly is 5% and 30%, respectively, have a poor myosin IIB mechanoresponse. As heavy chain phosphorylation inhibits myosin

IIB assembly, manipulation of the serine 1935 residue or PKCζ kinase, which phosphorylates this residue, is sufficient to shift the systems towards the 20% myosin IIB assembly, increasing its mechanoresponse (Fig. 2D) (Schiffhauer et al., 2019). This example illustrates how protein post-translational modification, phosphorylation in this case, can be used by cells to control the setpoint. In addition, the load-bearing capabilities of the structural proteins themselves can have impact at a distance, allowing force sharing between the proteins to be another means of tuning the setpoint.

Force sharing

The RacE-mediated tuning of myosin II mechanoresponsiveness discussed above suggested force sharing as a means of controlling the setpoint (Effler et al., 2006; Ren et al., 2009). Direct studies of the molecular entities that generate the foundational mechanics confirmed force sharing as a mechanism of distribution of forces across a cytoskeletal network (Luo et al., 2013). Traditionally a phenomenon studied in engineering, the concept of force sharing describes multiple components bearing the load of a system or network.

Because forces across the cortex are shared between the actin crosslinkers and myosin II, the more proteins across which to distribute the load, the less load each protein experiences. Furthermore, changes in the total amount of load-bearing proteins impact the amount of load experienced by the mechanoresponsive proteins, thereby affecting their ability to mechanorespond. For example, in *Dictyostelium*, the cross-linker α-actinin only displays mechanoresponsiveness in the absence of myosin II, suggesting that this crosslinker only experiences enough force to mechanorespond when myosin is not sharing load in the network. In addition, removal of some types of actin crosslinkers, including those which are not mechanoresponsive (e.g. dynacortin), reduces the amount of stress necessary to trigger a myosin II mechanoresponse (Luo et al., 2013). Thus, force sharing represents a systems-level determinant of setpoint positioning.

Once the forces are experienced by the crosslinkers, the actin-binding lifetime is modulated by the catch-slip characteristics of the crosslinker–actin bond. A catch-slip bond is a type of bond where low to moderate forces increase the binding lifetime and higher forces decrease binding lifetime. If the force surpasses a threshold, this decreases the binding affinity, causing the bond to fail (Chan and Odde, 2008). Thus, if the load is too widely distributed across the network, individual crosslinkers may not sense sufficient load to engage. Conversely, if there are too few crosslinkers sharing the load, the force of each of them may be too great, causing the crosslinkers to slip and release from the actin. Therefore, the catch-slip-bond nature of proteins predicts the scenario of a biphasic relationship between load and the ability of a protein to engage with the network, and an 'optimal' force that allows for proteins to mechanorespond.

Myosin II represents a special case where mechanical load triggers the myosin II to stall in the isometric transition state, which is also the cooperative binding state. In this state, the lifetime of binding to the actin filaments is increased and induces allosteric conformational changes along the actin filament that allows for cooperative binding of myosin motors and localized assembly of bipolar thick filaments (Mahajan et al., 1989; Veigel et al., 2003; Ren et al., 2009; Tokuraku et al., 2009; Uyeda et al., 2011; Luo et al., 2012). This behavior allows for the tension in the cytoskeletal network to be shared across many myosin motors. These motors can then maintain tension or remodel the network, relieving the stress. In fact, differences in the actin-binding duty ratio of non-muscle myosin II molecules likely contribute to different behaviors of the mammalian myosin II isoforms. For example, the higher duty ratio of non-muscle myosin IIB allows it to bear resistive strains over long-time scales (Kovacs et al., 2007; Nagy et al., 2013), thus allowing it to form stable structures at the rear of a migrating cell (Shutova et al., 2017).

In the context of cytokinesis, these force-sensitive properties of the crosslinkers and myosin II have unexpected consequences. Studies have found that depletion of actin crosslinkers and myosin II increases the velocity of cytokinesis furrow ingression during late stages of cytokinesis, whereas their overexpression can lead to cytokinetic failure (Robinson and Spudich, 2000; Zhang and Robinson, 2005; Mukhina et al., 2007; Kee et al., 2012; Descovich et al., 2018). This behavior is due to the interface between contractility, cortical viscoelasticity and the passive fluid mechanics of the furrow ingression process. Furthermore, as the network becomes loaded, the crosslinkers and motors bind more tightly, leading to a stiffer network (strain-stiffening mechanics; Glossary) (Reichl et al., 2008; Poirier et al., 2012; Srivastava and Robinson, 2015).

In addition, force sharing is visible in the context of super-diffusive behaviors of the cortex (Glossary). Much of the super-diffusive behaviors of the cell are lost in *Dictyostelium* cells with a myosin II heavy chain knockout (encoded by the *mhcA* gene; *myoII* null), and this super-diffusivity is not restored by an uncoupler myosin II mutant that has normal ATPase activity, but a short step size (Girard et al., 2006). These observations naturally implicate myosin II as the active component that drives fluidity (Glossary). However, depletion of actin crosslinker dynacortin in the *myoII*-null cells significantly restores their super-diffusive behavior, suggesting that myosin II activity promotes the active components of the cortex, but does not necessarily generate them on its own. In fact, multiple molecular components contribute to these dynamical phenomena and cell mechanics, and, in essence, one role of myosin II is to help 'stir the pot', antagonizing some of the cross-linkers and allowing these super-diffusive behaviors to emerge (Girard et al., 2006).

On a larger scale, measuring cortical flow behaviors in the *Caenorhabditis elegans* zygote has revealed the concept of morphogenetic degeneracy (Naganathan et al., 2018). Actin cortex structure and dynamics (including cortical flow velocities, chirality and myosin II foci) were quantified upon depletion of various actin-binding proteins and actomyosin regulators. Through dimensional analysis, certain proteins that had similar effects on cortical flow could be grouped into specific phenotypes, including changes in the chirality index and oscillations of cortical flow. The interpretation is that cortical mechanics are 'course-grained', allowing various components to contribute similarly to a single phenomenon, which on a molecular level may be explained by force sharing. Force sharing may then be a mechanism that generally provides robustness to shape change processes.

Complex assemblies

At this point, we have recognized that the contractile system is structured as a control system composed of feedback and tunable setpoints. The purpose of such a system is to be able to respond quickly to disturbances (inputs), thereby returning the system to its setpoint. This then begs the question as to how large molecular assemblies can be built on the 10–100 s timescale. The answer appears to lie in preformed complexes that exist in an equilibrium before their activation by spatial and temporal signals.

Precedence for this idea of complex assembly exists in *Schizosaccharomyces pombe* where precursor nodes are used to build the cytokinetic ring. Careful concentration measurements and

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super-resolution imaging of key cytokinetic proteins indicate that precursor structures form during mitotic entry, which are regulated by mitotic kinases, such as Cdr1p and Cdr2p (Vavylonis et al., 2008; Akamatsu et al., 2014, 2017). Upon mitotic entry, the sequential addition of Mid1 (anillin), Myo2 heavy and light chains, and Rng2 (IQGAP2) forms the nodes that then coalesce along the equatorial region to build the cytokinetic ring (Wu and Pollard, 2005; Akamatsu et al., 2014). Interestingly, these nodes initially form during interphase and coalesce to drive ring constriction. Their presence in interphase also suggests the potential for functions beyond cytokinesis (Martin and Berthelot-Grosjean, 2009; Moseley et al., 2009; Akamatsu et al., 2014).

Similarly, biochemical interactions between myosin II, cortexillin I and IQGAP2 allow the formation of assemblies within the cytoplasm during interphase in *Dictyostelium*. These so-called 'contractility kits' are preformed mechanoresponsive complexes that may subsequently accumulate at the cortex upon their activation by biochemical and/or mechanical signals (Fig. 3) (Kee et al., 2012; Kothari et al., 2019). While these kits appear to be critical during mechanoresponse in interphase cells, it is likely they are also the components that build the cytoskeletal meshwork at the equatorial region during cytokinesis to drive furrow ingression. This raises the intriguing question of whether these kits are functionally similar to the relatively stable cytokinetic nodes in fission yeast. It is possible that these stable assemblies (with minute timescale dynamics) appear within the lower concentrations of actin and cytokinetic proteins present in yeast, whereas much faster dynamics (on a timescale of seconds) are at play in *Dictyostelium* where their concentrations are also much higher (Wu and Pollard, 2005; Laporte et al., 2011; Robinson et al., 2012; Srivastava and Robinson, 2015).

The identification of such complexes in various systems will further enhance our understanding of how these assemblies contribute not only to cytokinesis, but also to more general shape-change events that occur during development. For example, in the *Drosophila* notum epithelium, jitterbug (filamin) and non-muscle myosin II were recently discovered to form a complex that is critical for tension and polarity maintenance during development of the flight muscle (Manieu et al., 2018).

Another example of complex assemblies combined with feedback control may be seen in the enhancement of actin filament assembly by a membrane-associated pool of actin monomers during actin polymerization. WASP family proteins are anchored at the membrane where they create a local high concentration of actin subunits, which are maintained in the form of profilin–actin where profilin sequesters and stimulates actin subunit nucleotide exchange. By creating this local high concentration, the actin polymerization is significantly accelerated (Bieling et al., 2018). Quantitative analysis of this mechanism for the regulation of actin polymerization demonstrates the ability of pre-formed clusters to control network architecture (Mullins et al., 2018). Because WASP-dependent actin polymerization requires both the presence of an actin filament and profilin-bound actin monomers, increased polymerization eventually limits the amount of available profilin–actin around the surface, generating an internal negative-feedback loop (Mullins et al., 2018). The presence of this feedback control reveals the importance of membrane-bound filament organization, rather than simply cytoplasmic polymerization as is traditionally depicted. This work has been further supported by a recent study exploring the stoichiometry-dependent control of actin assembly during liquid–liquid phase separation (Case et al., 2019). Complexes of phosphorylated nephrin, N-WASP and the Arp2/3 complex at the membrane allow for cluster formation, which increases Arp2/3

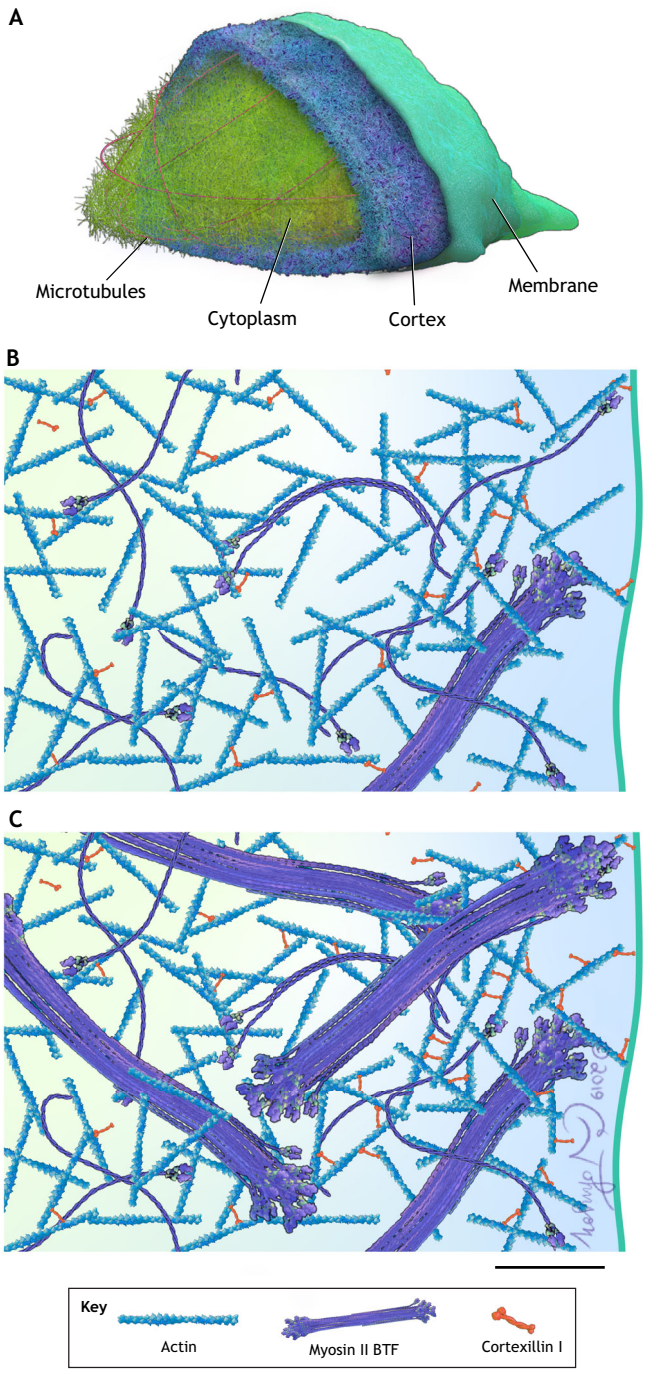

Fig. 3. Depictions of myosin II and cortexillin I assembly upon mechanical stress. (A) Diagram of a cell, including membrane, actin cortex, microtubules and cytoplasm. (B) Cortex, including actin, myosin II and cortexillin I, depicted before application of mechanical stress. Although these proteins exist in preformed contractility kits, their structures are currently unknown and are therefore not shown here. (C) Myosin II bipolar thick filaments and cortexillin I accumulate at the cortex as the network senses mechanical stress. Scale bar: 100 nm.

complex-mediated actin assembly and relies on the dwell-time of these proteins at the membrane. The formation of these clusters was observed both *in vitro* and in kidney podocytes and activated T cells, but it is likely that the underlying concepts are more universal, especially with regard to actin polymerization that drives protrusions during cell migration (Case et al., 2019). Such membrane–cortex

attachment is also critical for other processes, including clathrin-mediated endocytosis. Indeed, a recent study demonstrated that Myosin I (Myo5 in *Saccharomyces cerevisiae*) anchors the actin-polymerization factors Arp2/3 complex and WASP to the membrane to mediate its invagination during endocytosis (Pedersen and Drubin, 2019).

Feedback across long timescales

Feedback through mechanoresponsive proteins can occur within timescales from a few seconds to minutes, although the associated feedback loops also function over prolonged periods of time to integrate different cellular processes. Mechanically transduced transcription factors have emerged as key regulators of the feedback between the mechanical state of the cell and changes in gene expression to maintain homeostasis and cellular identity (Broders-Bondon et al., 2018; Salvi and DeMali, 2018; Kassianidou et al., 2019). Among these are the Hippo pathway proteins Yes-associated protein (YAP, also known as YAP1) and transcriptional coactivator with PDZ-motif (TAZ, also known as WWTR1), which regulate E-cadherin junctional organization, chromatin remodeling and even cell metabolism (Panciera et al., 2017). In fact, the Hippo pathway is known to be homologous to the septation initiation network (SIN) and mitotic exit network (MEN) in *S. pombe* and *S. cerevisiae*, respectively. These pathways coordinate chromosome segregation with cytokinesis through a sequence of signaling cascades through well-conserved kinases, their regulatory components and scaffolding proteins (Hergovich and Hemmings, 2012; Simanis, 2015). In mammalian systems, various Hippo pathway proteins have been shown to have cell cycle progression phenotypes (Yabuta et al., 2007; Hergovich and Hemmings, 2012).

Feedback between cell mechanics and YAP is critical for cell identity determination (Dupont et al., 2011). Studies have found that physiologically relevant mechanical cues, such as substrate stiffness, help dictate the differentiation of progenitor stem cells, and that myosin II plays a central role (Engler et al., 2006). Moreover, YAP translocation in and out of the nucleus in response to substrate stiffness is required for stem cells to differentiate accurately (Guilak et al., 2009; Lian et al., 2010; Smith et al., 2017; Totaro et al., 2017). This mechanically transduced transcriptional pathway also engages in positive feedback with the cytoskeletal network. YAP is not only a downstream effector of mechanical inputs, it also has a cell autonomous impact on cortical tension and works through TEAD to drive expression of key mechanical proteins, including myosin light chain (Bai et al., 2016). In addition, a recent study found that the chromatin remodeling SWI/SNF complex binds to YAP in the nucleus through ARID1A, which prevents the interaction between YAP and the transcription factor TEAD. This, in turn, prevents YAP-mediated progenitor-like properties in human mammary epithelial cells (HMECs) (Chang et al., 2018). Here, SWI/SNF functions as a negative regulator of YAP using sequestration of YAP to maintain differentiated states. Loss of ARID1A or SWI/SNF through genetic lesions may have decreased thresholds for YAP activation, leading to loss of differentiation in tumor cells (Chang et al., 2018). Similarly, the loss of negative regulation of YAP by myosin II allows for activation of pro-growth pathways in cancer cells and tumorigenesis (Picariello et al., 2019).

YAP/TAZ also generate a negative-feedback loop that is critical for maintaining persistent cell migration. YAP regulates myosin II phosphorylation through multiple compensatory pathways of myosin II light chain phosphorylation and modulates cell migration (Mason et al., 2019). Thus, while myosin II activity is critical for motility, increased activity is inhibitory to migration, highlighting the importance of both positive- and negative-feedback loops mediated by transcription factors to drive cellular processes. In this scenario, YAP/TAZ displays the function of both a sensor and an actuator that ultimately integrates with other feedback controllers like myosin II.

A number of recent studies have also explored the feedback between cell mechanics and metabolism. Actin dynamics affect mitochondrial fission and fusion, which in turn impacts mitochondrial function (Beck et al., 2012; Hatch et al., 2014). In addition, recruitment of mitochondria to the leading edge of a cell also promotes cell migration and invasion in tumor cells (Caino et al., 2015; Cunniff et al., 2016). Furthermore, the signaling between focal adhesions, integrins and metabolic enzymes also provide a possible means for feedback. For example, the application of shear stress or force on E-cadherin at cell–cell junctions results in the activation and recruitment of AMP-activated protein kinase (AMPK) to adhesion complexes, which results in increased phosphorylation and activation of myosin II. This also increases glucose intake and ATP production, providing the cell with increased energy to actively respond to the change in its environment (Bays et al., 2017; Salvi and DeMali, 2018).

Moreover, AMPK signaling drives changes in cortical tension that regulates entosis, the process whereby softer cells engulf stiffer cells, that is found in many solid tumor types and is likely a response to the nutrient deprivation in highly competitive growth conditions (Overholtzer et al., 2007; Sun et al., 2014; Hamann et al., 2017). In the breast cancer cell line MCF-7, glucose deprivation results in a bimodal distribution of cells, which are characterized by either a low or high elastic modulus. The increase in elastic modulus is driven by AMPK activation, highlighting the role of AMPK as a transducer of metabolic signals to cellular mechanics (Hamann et al., 2017). More recently, another study has demonstrated a myosin II-dependent increase in stiffness of brown/beige adipose tissue and isolated brown adipocytes in response to cold challenge (Tharp et al., 2018). Here, actomyosin contractility was critical for the thermogenic capacity of the cells and the induction of uncoupled respiration through YAP/TAZ-mediated gene regulation (Tharp et al., 2018). Therefore, these studies are beginning to reveal parts of the sophisticated systems that are in place to regulate cell mechanics in the context of other cellular processes.

Conclusions

The myosin II machinery, which includes actin, myosin II and actin crosslinkers, can no longer be viewed as a merely passive, downstream effector of upstream signal transduction networks. Contractility, often considered the main function of myosin II, is only one of its many roles. In reality, myosin II contributes in at least eight different ways to cell function: cortical tension, viscoelasticity, fluidization, mechanoresponse, membrane–cortex attachment, adhesion and feedback, in addition to contractility. More accurately, the myosin II network is a sophisticated integrator of mechanical and biochemical signaling inputs that continuously ‘senses’ and ‘monitors’ its status by using its control system structure in order to promote cell- and tissue-level morphogenetic processes. Furthermore, the myosin II contractile machinery communicates and integrates these inputs into changes on the longer timescale that are induced by directing the gene expression and metabolic profiles. Although we have focused on myosin II as a key player of the mechanobiome, many components identified in the control systems include elements that feed into signaling, growth and cell identity pathways. While it is necessary to consider the

broader concepts of genomics and epigenetics, proteomics and metabolomics, investigating the role the mechanobiome plays in integrating these 'omics' to drive the behavior of cells, tissue and ultimately organisms will be essential.

The impact of fusing control theory with the concepts of the mechanobiome has a wide range of benefits. Not only does this integration provide a framework for understanding how cells, and ultimately tissues, function, it provides the next generation of entry points for creating optimal therapeutic interventions. For example, one might want to pharmacologically target a key component (e.g. a non-muscle myosin II) to treat cancer (Ivkovic et al., 2012; Surcel et al., 2015; Wong et al., 2015; Picariello et al., 2019; Surcel and Robinson, 2019; Surcel et al., 2019). However, if the system is shifted one way or the other relative to its setpoint optimum, it would be possible to erroneously shift the system so that the disease scenario is exacerbated instead of corrected or improved. Another potential application might be found in cell engineering strategies. Cellular behaviors could be engineered more precisely if it is understood where the system is poised and in which direction it should be shifted. Finally, we hope to encourage broadening our tendency to consider linear pathways by incorporating the universal principles of control theory to create a truly systems-level understanding of biology.

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Competing interests
The authors declare no competing or financial interests.

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