Regulating cell shape during cytokinesis

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Summary. During cytokinesis, the last step in cell division, cells must rearrange their shape so as to produce two daughter cells of equal size. To this end, the temporal and spatial distribution of a wide variety of proteins must be coordinated. In this paper we review some of the basic steps in the process. Moreover, we argue that a key step in the process is the feedback regulation of motor proteins. In doing so, we show that the study of cellular shape change can benefit from a systems-level approach.

1 Introduction

The ability of a cell to change shape is crucial for the proper function of many cellular processes. For example, cells of the immune system migrate in response to pathogen infections by crawling, which involves cycles of extensions and contractions that deform their entire cell surface [1]. Similarly, during the last stage of cell division, known as cytokinesis, cells rearrange themselves so as to produce two identical daughter cells; see Fig. 1.

The ability to change cellular shape requires that the distribution of different classes of molecules be regulated both spatially and temporally. How these events are coordinated is understood only in broad terms, but appears to be a tightly regulated process. In this review we outline some of the features of this regulation, focusing on cytokinesis. We argue that cytokinesis is controlled by a mechanical feedback path between the cell shape and the localization of proteins that provide most of the actuation.

Understanding the principles of cytokinesis not only sheds light on how cytokinesis is regulated but also has broad implications for cell shape changes in other biological processes such as nuclear organization, gene expression, protein synthesis and cytoskeletal organization [2–4]. Cytokinesis is also essential for human health since the human body regulates ongoing basal levels of cell proliferation, which are required to replenish continuously the blood streams, skin, hair, absorptive epithelia of the gut and numerous other processes. This process is extremely important to humans since at any moment

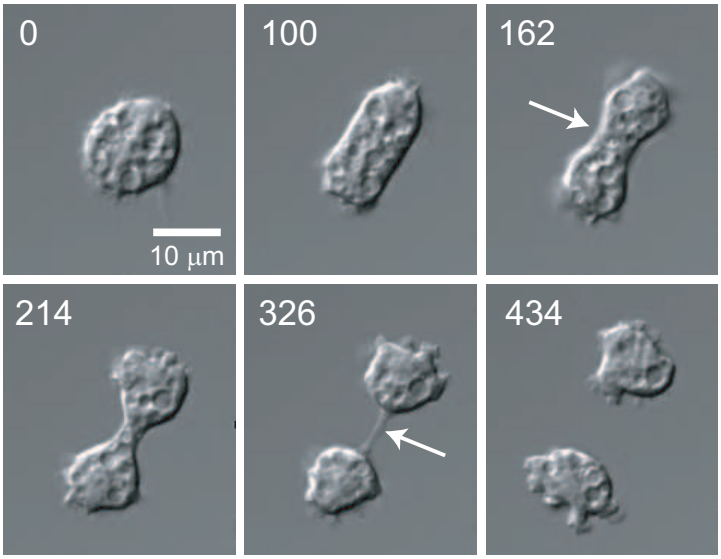

Fig. 1. Time series of a dividing *Dictyostelium* cell. The cytokinesis process can be separated into three phases; see Section 4.4. The numbers represent seconds after the first frame. During the first phase the cell elongates into a cylinder (0–162 s) and the furrow begins to ingress. During Phase 2 the cleavage furrow (white arrow at 162 s) continues to ingress (162–326 s). During Phase 3, the bridge (white arrow at 326 s) elongates leading to eventual daughter cell separation (434 s).

$\sim 10^8$–10^9 cytokinesis events are underway among the $\sim 10^{13}$ eukaryotic cells of the human body.

2 Biological background

Cytokinesis is the mechanical separation of a single mother cell into two daughter cells [5]. During this process, the mother cell rounds up and then elongates. A contractile ring forms at the middle of the cell and constricts until a thin cylindrical bridge connecting the two daughter cells is formed. This cylindrical bridge continues to thin until it severs, resulting in two separate daughter cells.

2.1 Stages of mitosis

Cytokinesis generally begins late in mitosis, the process of nuclear division, and continues until cellular division is achieved; see Fig. 2. Prior to mitosis, chromosomes that contain the cell's genetic information have been duplicated but are physically connected and contained within a single nucleus. For each

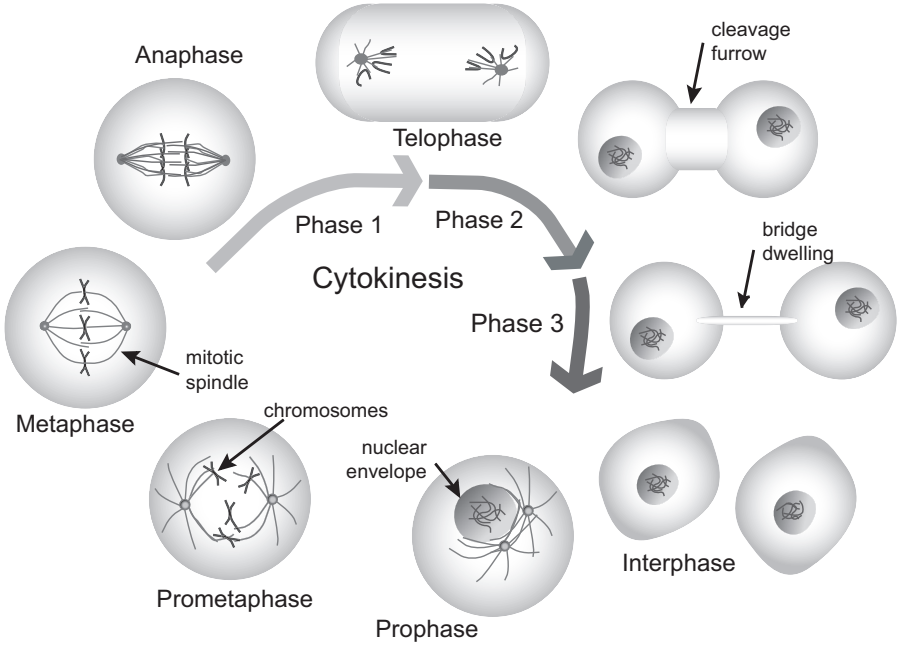

Fig. 2. Steps in the cell cycle. Cells spend most of their life in interphase, where DNA replication takes place. This is followed by nuclear division, known as mitosis. Mitosis consists of five sequential stages including prophase, prometaphase, metaphase, anaphase, and telophase. During prophase, chromosomes condense and the mitotic spindle starts to form. The mitotic spindle is composed of an array of microtubules, which are rigid hollow rods composed of tubulin subunits. Prometaphase consists of dissolution of the nuclear envelope, the barrier that separates the nucleus from the interior of the cell. As a result of nuclear envelope breakdown, spindle microtubules are able to enter the nuclear region and bind to kinetochores attached to centromeres, which constitute regions where replicated chromosomes are joined. In some organisms, such as *Dictyostelium*, the nuclear envelope does not break down, but instead, becomes porous. During metaphase, chromosomes become aligned at the metaphase plate, which is an imaginary plane positioned at a right angle between the spindle poles. In anaphase, the chromosomes start to separate due to forces generated by the mitotic spindle. Finally, during telophase, chromosomes reach opposite ends of the cell, the nuclear envelope reforms and chromatin decondenses. Cytokinesis begins prior to anaphase and completes following mitosis. The mitotic spindle disassembles during cytokinesis, after the completion of mitosis. The phases of cytokinesis are explained in Section 4.4.

daughter cell to receive an equivalent set of genetic material, the replicated chromosomes must be physically separated and encapsulated within separate nuclei positioned at opposite ends of the cell prior to the completion of cytokinesis.

3 Key molecular players in cytokinesis

Across different species, a couple hundred proteins have been identified as playing a variety of roles during cytokinesis. Here, we will focus only on central players that are known to affect the mechanical aspects of cytokinesis directly [6] with a particular emphasis on the components found in the social amoeba *Dictyostelium discoideum*.

In most organisms, the cytoskeleton is composed of three types of filaments: actin, microtubules, and intermediate filaments. Some organisms such as *Dictyostelium* do not contain cytoplasmic intermediate filaments. Both actin and microtubules polymerize from individual subunits (monomers) into filament form. Once in filament form, these molecules play crucial roles in cytokinesis.

The actin filament network contributes structure and support for the cell and is the major cytoskeletal system that drives cytokinesis [6, 7]. However, the microtubule network, which is primarily responsible for chromosomal separation, delivers cytokinesis regulatory cues to the actin network, and bidirectional communication exists between the two polymer networks [8, 9]. Here, we will primarily discuss the actin-based network of the cell.

3.1 Actin

Actin is a globular protein that uses energy from ATP hydrolysis to assemble into semiflexible rod-like filaments [7]. Actin filaments have the structure of a two-start right handed helix that spans 36 nm for every 13 actin monomers; thus, every new actin monomer extends the filament a distance of 2.7 nm [10].

By drawing upon the energy from ATP hydrolysis at a given concentration of actin monomers, actin polymerization can generate pN-scale forces while lengthening the actin filament. Since the γ-phosphate of a single molecule of ATP stores $\sim 100\,\mathrm{pN{\cdot}nm}$ of energy and given the 1–2 pN of force that has been measured for actin polymerization, the efficiency of the actin-based force generation is of the order of 3–5% [11]. However, at cellular concentrations of monomeric actin and with associated proteins that may increase the flexural rigidity of the polymer, it is quite possible for the actin machinery to generate significantly greater forces during polymerization *in vivo*.

Actin dynamics. Actin filaments are polymerized from a pool of monomers following a reaction mechanism whose parameters depend on the nucleotide state of the actin monomers. Thus, a number of regulatory mechanisms, and therefore actin-associated proteins, have evolved to govern and regulate the assembly and higher order organization of actin filaments [12]. Regulatory activities include actin filament nucleation (Arp2/3, formin), nucleotide exchange (profilin), monomer sequestration (profilin, β-thymosin), actin filament capping (capping protein), actin filament stabilization (tropomyosin, dynacortin), actin filament severing (cofilin) and actin filament crosslinking

(dynacortin, cortexillin, fimbrin). In living cells, many of these proteins have been implicated in cytokinesis in one or more biological systems, ranging from amoebas and yeasts to humans.

Actin polymer dynamics are also critically important for furrow ingression [13–18]. Depending on the organism, new actin filaments can be assembled in the furrow cortex or new filaments are recruited by flow along the plasma membrane. Cofilin, an actin severing protein, is required for actin filament turnover in the cleavage furrow [18]. In mammalian cells, myosin-II activity also appears to be required for normal removal of actin from the furrow cortex [19]. Myosin-II mechanochemistry may pull actin filaments from bundles exposing sites for cofilin to sever the actin. Thus, actin dynamics are controlled at a variety of levels.

3.2 Myosin-II

Central to cytokinesis, myosin-II is responsible for much of the net forces of cytokinesis [5, 20, 21]. Filaments of myosin-II bind to an actin substrate, also in filament form. Myosins are actin-activated motors that convert the chemical energy stored in the γ-phosphate of ATP into mechanical work [22]. This energy conversion is carried out by each myosin's motor domain, which undergoes a conformational change to generate force ($\sim 3\,\mathrm{pN}$ for myosin-II) while taking a step ($\sim 10\,\mathrm{nm}$ for myosin-II), to translocate its associated actin filament [23]. Thus, myosin-II is approximately 30% efficient in its mechanochemical transduction.

Myosin-II monomers, which actually consist of two heavy chains each with two light chains bound, assemble into bipolar thick filaments consisting of 10–400 monomers [24–26]. Myosin-II is typically considered a low duty ratio enzyme where each motor domain spends a fraction of its ATPase cycle in the force-generating state. However, the specific duty ratio can range from 1–80% [27–30]. Thus, as few as one or as many as tens of heads can be in the force-generating state at any point in time.

Myosin-II regulation. Significantly for cytokinesis in *Dictyostelium*, thick filament assembly is highly regulated. Phosphorylation promotes disassembly of the thick filaments while dephosphorylation promotes assembly [31]. This thick filament assembly regulation controls the amounts of myosin-II sent to the cleavage furrow cortex [21, 32]. Mutant myosin-II filaments that are non-phosphorylatable over-accumulate in the cleavage furrow cortex relative to wild type levels and at least one myosin heavy chain kinase, an enzyme that catalyzes phosphorylation reactions, is recruited to the cleavage furrow late in cytokinesis, presumably to drive disassembly of the myosin-II in preparation for final bridge scission [33].

3.3 Actin crosslinking proteins

Actin crosslinking has an integral role in regulating cytokinesis dynamics. Actin crosslinkers organize the actin and increase the mechanical resistance of the network by linking individual filaments to each other in the network. Pure actin networks generate some mechanical resistance owing to entanglements, salt-bridging, and the fact that the actin polymers are semiflexible, having a persistence length of $\sim 15\,\mathrm{nm}$. However, in *in vitro* systems, the presence of crosslinkers at varying stoichiometries with actin can vary the mechanical resistance of the actin network by greater than three orders-of-magnitude [7, 34–39]. *In vivo*, we have only detected a little over one decade of mechanical variation, ranging from latrunculin-treatment (which prevents polymerization of actin monomers) to overexpression of actin crosslinking proteins [40]. Thus, the allowable range for cytoskeletal resistance compatible with viability may be much narrower than is achievable *in vitro*.

Several actin crosslinking proteins have been implicated in cytokinesis either by localization to the cleavage furrow cortex or implicated using genetics. In *Dictyostelium*, the cortexillins are actin crosslinking proteins that are specifically recruited to the cleavage furrow cortex [41–44]. Cortexillins are required to maintain normal cleavage furrow morphology under both adhesive and non-adhesive conditions, indicating that they may play a more fundamental role in cytokinesis.

Dynacortin is an actin crosslinker distributed around the cell cortex and excluded from the cleavage furrow; this was the first indication that both global and equatorial cortices have genetically separable mechanical roles during cytokinesis [45, 46]. Dynacortin is a dimeric actin crosslinker, identified in *Dictyostelium*. Both cortexillin and dynacortin are required for wild type levels of cortical viscoelasticity during interphase [40].

3.4 Rho-superfamily members

Rho-superfamily proteins act as molecular switches to regulate cytoskeletal components. In mammals, two classes of this superfamily, Rac and Rho, have essential roles during cytokinesis and are both localized to the plasma membrane [47–49]. Rho is specifically activated in the cleavage furrow cortex by proteins localized to the interzonal microtubules of the central spindle. Inhibition of Rho leads to a failure to assemble a contractile ring properly. Rac appears to act globally and inhibition of Rac leads to furrowing of the global cortex [50].

In *Dictyostelium*, no clear Rho homologs can be found in the genome, rather there are several Rac-subfamily genes that have varying degrees of similarity to Rac and Rho. Instead, the Rho function appears to be provided by Rac1 and inhibition of Rac1 leads to failure to properly recruit the cortexillins to the cleavage furrow [51]. The *RacE* gene product is globally distributed during cytokinesis. Deletion of *RacE* leads to a severe defect ($\sim 20\%$ of wild type

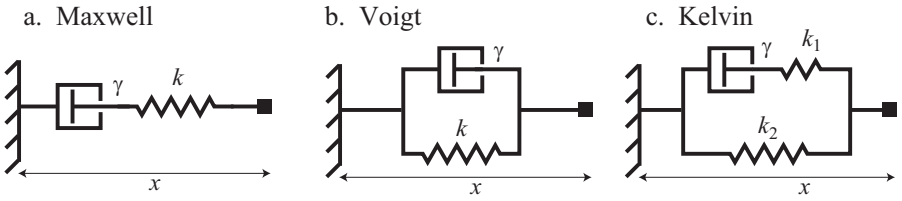

Fig. 3. Traditional mechanical models for cell deformation. The corresponding transfer functions between force and position are given in Table 1.

levels) in cortical mechanics and cytokinesis is defective under nonadherent conditions [52, 53]. The *RacE* mutant has normal filamentous actin levels but is defective in recruiting dynacortin to the cell cortex [45, 53]. Thus, the cortical mechanical defect of the *RacE* mutant is most likely due to a reduction in the actin crosslinkers. However, dynacortin is unlikely to be the only protein that is regulated by RacE. Further, removal of RacE and dynacortin from cells has synergistic effects on the dynamics of cleavage furrow ingression [20].

4 Mechanical nature of the cell

To study cell shape changes requires that we have a description of the underlying mechanical properties of the cell. Cellular shape is primarily dictated by the forces acting on the cortex and cytoplasm, which is usually viewed as a viscoelastic material; that is, it exhibits properties of both viscous liquids and elastic solids [1, 7]. This behavior can be observed in the time-dependent deformation response to mechanical stresses.

Mechanical models of material behavior are traditionally based on combinations of idealized linear spring and dashpot elements [7]; see Fig. 3. Determining the response of a system to induced stresses is a relatively straightforward application of linear system theory. Maxwell, Voigt and Kelvin bodies differ as to their composition. For example, the deformation induced by an applied force on a Maxwell body (Fig. 3a) is described by the differential equation:

$$\dot{x} = \frac{1}{k}\dot{f} + \frac{1}{\gamma}f$$

where we have used the fact that the force on the spring and dashpot are equal, and that the total deformation is the sum of the individual terms. In the Laplace domain, the ratio

$$G(s) := \frac{F(s)}{X(s)} = \frac{ks}{s + k/\gamma}$$

is known in the field as the *viscoelastic impedance* or, when $s = j\omega$, as the *complex viscoelastic modulus*. Note that it is common to express and plot $G(j\omega)$ as a function of frequency in log-log form, as in Bode plots, but this is frequently done in Cartesian form

$$G(j\omega) = G'(j\omega) + jG''(j\omega)$$

rather than in polar form. In this case $G'(j\omega)$ and $G''(j\omega)$ are known as the *storage* and *loss* moduli respectively.

One common means of studying the mechanical properties of cells is by applying a step change in the force and observing the ensuing cellular deformation, known as the *creep compliance*:

$$\Gamma(t) = x(t)\Big|_{f(t)=\text{“unit step”}} = \mathcal{L}^{-1}\left[\frac{1}{sG(s)}\right]. \tag{1}$$

Alternatively, one may apply a deformation and measure the *relaxation modulus*; that is, the force that must be applied to maintain a unit-length deformation. This equals[3]

$$\Upsilon(t) = f(t)\big|_{x(t)=\text{“unit step”}} = \mathcal{L}^{-1}\left[\frac{1}{s}G(s)\right].$$

Based on time series analysis of the creep or relaxation responses, it is possible to obtain estimates for the viscoelastic properties of the cell. Note that, because of the presence of a pole and a zero in $G(s)$ for the Kelvin body, both responses exhibit exponential decays. In contrast, the Maxwell and Voigt bodies only exhibit an exponential decay in either the creep (Voigt) or relaxation (Maxwell) responses.

For more complicated responses, time-domain analysis is difficult to perform. Several other techniques are currently used to measure the frequency-dependent rheological properties of cells.

The mechanical properties of biological polymers can be studied by mechanically stimulating *in vitro* samples at different frequencies by external harmonic excitation [35, 54]. One mechanism for doing so is the cone-plate rheometer, where actin gels are placed between a cone and plate. The plate is rotated at a given frequency thereby inducing a rotation of the cone that can be measured to obtain the complex viscoelastic modulus, assuming that

$$x(t) = G(j\omega)e^{j\omega t}.$$

For live cells, more passive methods are also employed [55–58]. One technique, referred to as microrheology, is based on placing small beads at the surface of the cell and tracking their mean-squared-displacement. Assuming

[3] Note that the usual notation for the relaxation modulus is $G(t)$. We will eschew this notation to avoid the obvious confusion with $G(s)$.

Type	$F(s)/X(s)$	Creep Function $(x(t))$
Spring	k	$1/k$
Dashpot	γs	t/γ
Maxwell	$\dfrac{ks}{s+k/\gamma}$	$1/k + t/\gamma$
Voigt	$k+\gamma s$	$\dfrac{1}{k}\left(1-e^{-kt/\gamma}\right)$
Kelvin	$(k_1+k_2)\dfrac{s+(k_1 \parallel k_2)/\gamma}{s+k_1/\gamma}$	$\dfrac{1}{k_1+k_2}\left(\dfrac{k_1}{\gamma}+\left(1-\dfrac{k_1}{\gamma}\right)e^{-(k_1 \parallel k_2)t/\gamma}\right)$

Table 1. Mechanical properties for different viscoelastic models of cells. We have used the notation $k_1 \parallel k_2 = k_1k_2/(k_1+k_2)$.

that motion is due to Brownian motion, the mean-square displacement of a particle diffusing in a three-dimensional medium equals

$$\langle x^2(t)\rangle = 6Dt$$

where D is the diffusion coefficient [59]. Using the Einstein relation ($D\gamma = k_BT$) leads to

$$\langle x^2(t)\rangle = \frac{6k_BT}{\gamma}t$$

where γ is the coefficient of drag. For a spherical particle flowing in a viscous medium the drag coefficient is given by Stokes theorem: $\gamma = 6\pi\eta r$, where r is the particle's radius and η the viscosity of the medium. For a viscoelastic medium we replace η with $G(s)$ to obtain:

$$\mathcal{L}[\langle x^2(t)\rangle] = \frac{k_BT}{\pi rsG(s)}.$$

This is known as the Generalized Stokes-Einstein Relation (GSER). Note that, from (1) we find a link between the macro- and microrheological measurements:

$$\langle x^2(t)\rangle = \frac{k_BT}{\pi r}\Gamma(t).$$

4.1 Viscoelastic properties of cross-linked actin networks

Crosslinking proteins form dynamic associations, meaning that the kinetic rates for association and dissociation determine the mechanical properties of the cortex. For cross-linked polymer networks, the viscoelastic modulus is given by

$$G(s) = \frac{as}{s+k} \tag{2}$$

where k is the dissociation constant and a is the stiffness if the network were permanently crosslinked [38].

In our experiments, the observed frequency dependent viscoelastic modulus agrees well with this model for a wide-variety of genetic mutants for frequencies between 1–1000 rad/s (our unpublished data). At higher frequencies, however, the viscoelastic modulus appears to increase as $|G(j\omega)| \approx \omega^{3/2}$ which is consistent with some models of polymer dynamics [60]. Interestingly, several mutants we have considered do not follow the Maxwell model of (2), but instead have frequency-dependent viscoelastic moduli that do not fit a lumped-parameter model. System identification techniques, including those developed by Keith Glover [61], will be invaluable in generating mechanical models of the mechanical properties of these cells.

Quantitative analysis and analytical modeling of furrow dynamics of wild type and several genetically modified strains indicate that the viscoelastic properties of the equatorial and global cortices have distinct and significant roles in controlling cytokinesis dynamics as well as distinct mechanical properties [20].

4.2 Viscoelastic model of the cell

Three general classes of models for cellular mechanics have been proposed; see [6] for a review. We focus on the *cortical shell-liquid core* model. This model assumes that the cell is composed of a cortical shell composed of membrane, actin cytoskeleton and associated cross-linking proteins and can be modeled using a Maxwell element as in (2). Inside the shell is a liquid core composed of viscoelastic cytoplasm where the viscosity is force-dependent. Furthermore, we assume that the cell is much larger than its individual components, so that a continuum assumption is valid. Using this model and level-set methods we have begun to simulate cytokinesis (article in preparation).

4.3 The Yoneda-Dan model of cytokinesis

Yoneda and Dan suggested a model for describing the balance of forces at work during cytokinesis [21, 62]. In their model, the minimal contractile force required for stabilizing each of these intermediate shapes is proportional to the global steady state stiffness of the cell and is dependent on the extent of furrow ingression; see Fig. 4.

Assuming that cellular volume is conserved during cytokinesis, we can compute a formula for the radius of the cleavage furrow (r_f) as a function of the angle θ. Let r be the radius of the mother cell just before commencing cytokinesis and $r'(\theta)$ be the radius of the two daughter cells as a function of the angle θ. The total volume of the dividing cell is

$$\frac{4\pi}{3}[r'(\theta)]^3 + 2\pi[r'(\theta)]^3 \cos\theta(1 - \tfrac{1}{3}\cos^2\theta)$$

and this must equal $4\pi r^3/3$. This means that the ratio of cell radii satisfies:

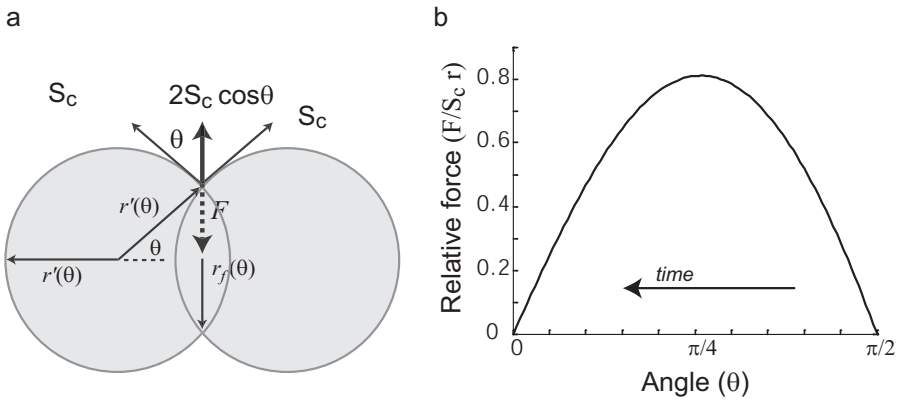

Fig. 4. Yoneda and Dan model of force distribution during cytokinesis. a) The force required to ingress the furrow, F, must counterbalance the pull from the stretch modulus (S_c). The radius of the two daughter cells equals r'; that of the furrow r_f. b) The force distribution as a function of the angle θ.

$$\frac{r'(\theta)}{r} = \left(1 + \tfrac{1}{2}\cos\theta(3 - \cos^2\theta)\right)^{-1/3}$$

Using the Yoneda-Dan equation for computing the force needed to divide the cell yields

$$\begin{aligned} F &= 2r_f S_c \cos\theta \\ &= 2S_c r'(\theta)\sin\theta\cos\theta \\ &= S_c r \frac{\sin 2\theta}{\left(1 + \tfrac{1}{2}\cos\theta(3 - \cos^2\theta)\right)^{1/3}} \end{aligned}$$

Here S_c represents the stretch modulus (also referred to as surface tension), which is the viscoelasticity in the plane of the cell cortex. A plot of the function $F/S_c r$, as a function of θ shows that the required force to stabilize the shape is biphasic; see Fig. 4b.

Using the Yoneda and Dan model, the amounts of myosin-II recruited to the cleavage furrow cortex can be predicted by considering biophysical properties of myosin-II [21, 63]. Measured amounts of myosin-II sent to the cleavage furrow cortex agree closely with the amount predicted, suggesting that cells regulate myosin-II localization to accomplish cytokinesis [21]. However, given the observed geometry, this model should only accurately apply when the curvature of the furrow is continuous with the emerging daughter cells.

4.4 Stages in cytokinesis: different phases

In *Dictyostelium*, cytokinesis proceeds via a highly stereotypical geometry, which can be subdivided into three phases [6, 20]. Each phase is regulated by different subsets of molecules.

During Phase 1, the mother cell rounds up, elongates into a cylinder and the furrow begins to ingress while maintaining continuous curvature with the two daughter cells. In Fig. 1, this corresponds to the first 100 s. This stage appears to be dependent upon myosin-II, which is recruited to the cleavage furrow cortex during this stage, since *Dictyostelium* cells that lack myosin-II grown under nonadhesive conditions undergo cell rounding, but fail to elongate, constrict and divide [64].

During Phase 2, the cleavage furrow continues to ingress and forms an intracellular bridge with a cylindrical shape connecting the two daughter cells [20]. The diameter of the cylindrical bridge decreases approximately 50-fold while lengthening only 2–4 fold, pushing cytoplasm out of the bridge. Genetic perturbations have revealed that this process is regulated in part by actin crosslinking proteins, which impart mechanical resilience to the cytoskeleton. Specifically, genetic inhibition of dynacortin and RacE reveal a substantial increase in the rate of cylindrical furrow thinning. During this stage, myosin-II reaches a maximum concentration at the cleavage furrow and then begins to decrease. The Yoneda and Dan model can explain Phase 1. However, during Phase 2 the cylindrical bridge has discontinuous curvature with the two daughter cells so that a different scheme is required to account for the mechanics of the cell.

To address the issue of how furrow thinning is governed, a cylinder thinning model has been developed for *Dictyostelium* cytokinesis [20]. In this model, four principal mechanical parameters (active radial stress, stretch modulus, viscosity and an axial compressive stress) are proposed to govern cytokinesis. By combining genetics, live-cell imaging, and biochemistry with modeling, each model parameter was able to be given a molecular identity. From the analysis, each genetic strain was shown to have a unique furrow-thinning trajectory. Wild type furrows thin following an exponential decay, while many of the mutant strains follow nonlinear, non-exponential dynamics. The wild type exponential trajectory is surprising since viscoelastic fluids are predicted to thin with a linear trajectory, suggesting that a braking mechanism slows the wild type furrows and converts the trajectory into an exponential decay. RacE and dynacortin provide the brake that slows cytokinesis and myosin-II acts as an accelerator [20]. Thus, it may be possible that the cell can regulate cytokinesis dynamics in part by modulating RacE and dynacortin activity, thereby allowing it to respond to environmentally induced stress.

Phase 3 is the bridge dwelling phase. It is genetically and quantitatively separable from Phases 1 and 2 [20]. During this phase the bridge does not thin appreciably and appears to be regulated by cytoskeletal disassembly.

This series of shape changes has important implications for the mechanics of cytokinesis. First, Phase 1 can be thought of as a movement away from an equilibrium shape while Phase 2 is a return to an equilibrium shape. Thus, a mechanical transition from an active process requiring force generation from myosin-II to a passive-like process is suggested.

5 Controlling mechanics of cytokinesis

Cytokinesis mechanics are regulated by the complex interplay between multiple concurrent processes including myosin-II mechanochemistry and localization, actin dynamics, actin crosslinking proteins, and molecular switches of the Rho-superfamily. How do these various processes fit together spatially and temporally to regulate this complex process?

Microtubules provide cues which initiate cytokinesis. Interzonal microtubules positioned at the midpoint of the cell act as tracks through which the components of the contractile rings are recruited. Rac1 recruits the cortexillins to the cleavage furrow, while RacE recruits dynacortin to the global cortex and globally inhibits contraction.

During Phase 1, the cell rounds up, elongates and furrow ingression begins. At a molecular level, myosin-II and cortexillin-I are localized to the furrow, while dynacortin is excluded from the furrow and enriched in the polar cortex. New actin filaments are also formed at the furrow region, and some studies suggest that actin polymerization at the two polar cortices may provide forces which help drive elongation.

During Phase 2, thinning of the cleavage furrow to a narrow cylindrical bridge, myosin-II reaches its peak accumulation at the furrow and then decreases. Myosin-II generates contractile forces to drive ingression, but may also play a role in removal of actin from the furrow cortex [19]. Myosin-II mechanochemistry may pull actin filaments from bundles, exposing sites for cofilin, which is recruited to the furrow cortex, to sever the actin. As the furrow thins, myosin-II heavy chain kinase is recruited to the furrow late in Phase 2, driving disassembly of myosin-II filaments [33]. Cytokinesis concludes with disassembly of the thin bridge connecting the two daughter cells.

6 Role of mechanical feedback

Understanding how cells perform complex processes under a diverse array of conditions with high fidelity is an important question in cell biology. Feedback control systems are well appreciated in numerous biosynthetic and metabolic pathways. For example, the well studied glucose homeostasis pathway maintains a constant level of glucose in the bloodstream by employing negative feedback. The mechanochemical feedback system of muscle contraction has also historically been well studied [65–67]. Muscle contraction requires the

actomyosin system of the sarcomere (a single contractile unit of muscle) to generate force. The amount of force that is generated changes in response to the applied load. The myosin-II mechanoenzyme responds to applied loads by a strain-induced alteration of its mechanochemical cycle — namely, decreasing the ADP-release rate, which increases the amount of time the motor remains bound to the actin filament. This decrease in a single kinetic step increases the number of motor heads in the force-generating state, allowing each sarcomere to generate greater tension. Thus, in muscle, mechanical feedback acts through a direct modification of the mechanochemical coupling of the force-generating enzyme.

Evidence in support of nonmuscle cells having mechanical feedback mechanisms also exists. A number of studies have demonstrated that applied stresses change the biochemical makeup of the cortical cytoskeleton and lead to activation of regulatory kinases [68–70]. Cells also respond with cytoskeletal alterations to environmental stresses such as osmotic shock. In *Dictyostelium*, osmotic stress leads to a dramatic recruitment of myosin-II to the cortex and *myosin-II* mutant cells are significantly more sensitive to osmotic pressure [71].

6.1 Evidence for mechanical feedback in cytokinesis

Dividing cells may also have feedback mechanisms that allow them to successfully complete cytokinesis in diverse mechanical environments. By sensing when cytokinesis is not progressing properly and responding by making mechanical adjustments to correct the process, cells may utilize force-feedback mechanisms to control the amount of force that is exerted by the cleavage furrow cortex, possibly by regulating myosin-II accumulation.

Supporting evidence comes from studies in which *Dictyostelium* cytokinesis has been compared between three growth conditions: on surfaces alone, on surfaces with a sheet of agar overlaying the cells, and in suspension culture. In *Dictyostelium*, myosin-II is not required when the cells are propagated on surfaces, which allows for traction or adhesive forces to assist in cytokinesis; however, myosin-II is essential for non-adherent cytokinesis [64, 72] as well as under agar overlay [73]. By compressing the cell, the sheet of agar may increase the tension on the cytoskeleton, increasing the resistance so that additional force is required for furrow contractility. Consistent with this hypothesis, more myosin-II appears to accumulate in the cleavage furrow cortex of wild type cells sandwiched under a sheet of agar than when cells are grown on surfaces [73]. This suggests that the cell responds to mechanical perturbation by accumulating more myosin-II in the cleavage furrow, enabling successful completion of cytokinesis. Phase 1, in which the cell transforms from a spherical to a elongated cylindrical shape, utilizes the accumulation of myosin-II at the cleavage furrow to drive this shape change.

In Phase 2, the concentration of myosin-II reaches a peak and then decreases, with myosin-II phosphorylation thought to play a role in myosin-II

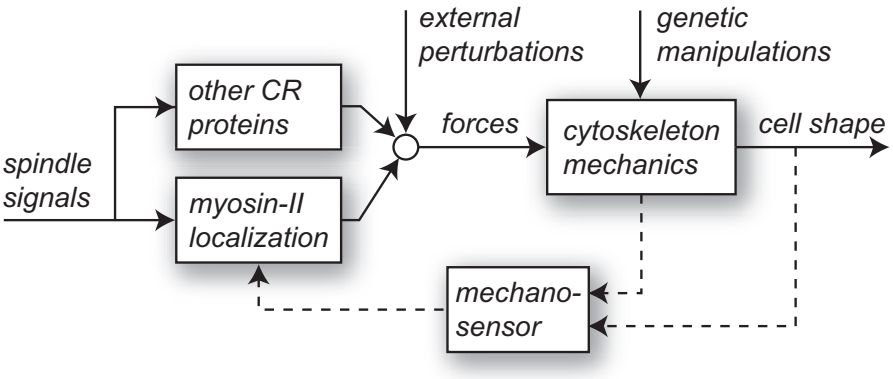

Fig. 5. Feedback model for regulation during cytokinesis. This block diagram captures our view of the mechanical regulation taking place during cytokinesis. Protein localization signals from the mitotic spindle polarize the cell. Together they help generate the forces that act on the cell mechanics and generate cell shape. We conjecture that a mechanosensor feeds back signals that correlate either to cell mechanics (such as cortical tension) or cell shape to influence the spatial localization of myosin-II (dotted lines) or other contractile ring (CR) proteins.

disassembly. Intriguingly, mutant myosin-IIs resistant to this regulation accumulate to higher levels in the cleavage furrow cortex suggesting not only a regulatory role for phosphorylation, but also illustrating that myosin accumulation in the furrow is a highly dynamic process [21]. Maintaining a myosin concentration under conditions of high turnover likely incorporates a sensory mechanism capable of detecting shape along with feedback mechanisms regulating progression through various mechanical stages. Since cells can complete cytokinesis with high efficiency in suspension culture or on surfaces (*Dictyostelium*) or in tissues or blood streams (mammalian cells) where they often experience compressive forces, mechanical feedback mechanisms may prove to be the essential principle that allows for successful cytokinesis.

6.2 Evaluating models of mechanical feedback

To elucidate the complex regulation of cytokinesis, two methods of perturbing the system are available; see Fig. 5. The transfer function of the cell can be modified by genetic perturbation. The cortical stretch modulus can be reduced by genetically removing the genes that regulate or encode for actin cross-linking proteins. For example, a genetic strain with a compromised cortical stretch modulus may require less force (since there is less cortical resistance) for cleavage furrow ingression. The second method of perturbing the system is by applying an external mechanical force. The cell's ability to reject this mechanical disturbance can be measured by changes in localization and

concentration of proteins. For instance, force may be applied to impede cytokinesis mechanically, which requires an increase in contractile force at the cleavage furrow to drive ingression. Combining these two methods, it is possible to probe for the existence of mechanical sensors that regulate the amount of force produced based on the mechanical conditions the cell experiences.

7 Conclusions

Cytokinesis involves a complex, dynamic interplay between biochemistry and cell mechanics. Its understanding has potential to shed light on a number of processes dependent on cell shape and may provide insight into numerous genetic disorders and tumor cell biology, as well as identify new therapeutic targets.

Cytokinetic failure results in binucleate cells, which increases the number of single sets of chromosomes. Accompanying this increase is the amplification of the centrosomes, which lead to multipolar spindles [74]. Subsequent errors in chromosomal segregation can also lead to aneuploidy, the state of having an incorrect number of chromosomes or chromosome sets, which is associated with many types of tumors and is correlated with tumor type and disease progression [75–77].

Cytokinesis also provides a source of novel potential drug targets that may be useful for the treatment of hyperproliferative diseases such as cancer. Taxol, a leading anti-cancer agent, targets mitosis by stabilizing microtubules, thereby inhibiting cell proliferation. However, because taxol inhibits a wide-range of microtubule functions, many microtubule-dependent processes are affected, leading to a number of side-effects, such as peripheral neuropathies. Investigators hope that unwanted side-effects can be avoided by specifically targeting dividing cells. Thus, chemical approaches are underway in a number of venues to identify novel mitosis and cytokinesis inhibitors; see for example [78].

Inextricably linked to cell mechanics, cytokinesis is a slowly evolving intrinsic cell shape change that depends on the cell's biochemistry to manipulate its fundamental physical properties.

The regulation of cell shape during cytokinesis is a complex biological process, and its complete elucidation requires knowledge from a variety of fields including mechanics, biophysics, genetics and cell biology. Moreover, because of the tight regulatory feedback paths that exist, the study of cytokinesis can greatly benefit by the application of control theory as other systems have, such as bacterial [79] and eukaryotic chemotaxis [80, 81], heat-shock regulation [82], cell polarity [83] and cell cycle regulation [84]. We believe that this presents a new frontier for the field of control engineering, a fact that has not gone unnoticed by Keith Glover who, as head of the Cambridge Engineering Department, selected "engineering for life sciences" as one the central

research themes for the department. To Keith we dedicate this paper on his 60th birthday.

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