ELSEVIER

Mechanics and regulation of cytokinesis

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Recent advances are revealing quantitative aspects of cytokinesis. Further, genetic analyses and cell imaging are providing insights into the molecular dynamics of cleavage furrow ingression as well as further refining our knowledge of the zones of the mitotic spindle that regulate the contractile properties of the overlying cortex. Ultimately, however, cortical mechanics are the result of signals that emanate from the mitotic spindle. A genuine quantitative understanding of cytokinesis must include a thorough analysis of the mechanical properties of the cortex and how signals modify these properties to dictate a well-controlled, error-free cytokinesis.

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Abbreviations

Arp2/3	actin-related protein 2/3
F	Force
FRET	fluorescence resonance energy transfer
MHCK	myosin-II heavy chain kinase
MKLP	mitotic kinesin-like protein
MLCK	myosin-II light chain kinase
PCH	*pombe* Cdc15 homology
PLK1	Polo-like kinase 1
R_f	furrow radius
S_c	cortical stretch modulus
UCS	UNC-45/CRO1/SHE4

Introduction

Cytokinesis is the mechanical process that cleaves the mother cell into two daughter cells at the end of the cell cycle. During cytokinesis, the cell undergoes a series of stereotypical shape changes (Figure 1). The cell first rounds up into a nearly spherical mother cell and then elongates forming a cylindrical shape. A furrow begins to ingress but maintains continuous curvature with the global cortex. A discrete bridge forms that appears as a cylinder connecting the two daughter cells; the curvature of the bridge is discontinuous with the curvature of the two daughter cells. The bridge thins, lengthens and is ultimately severed, producing two daughter cells.

These basic steps are nearly ubiquitous among cell divisions of metazoan and ameboid organisms. Because cytokinesis is so highly regimented geometrically, it is an ideal model cell shape change for studies of cell mechanics and morphogenesis and naturally highlights the function of several cytoskeletal systems. The microtubule network and its associated proteins coordinate the separation of cytoplasmic and genetic material and serve as key regulators of the overlying cortical actin cytoskeleton, which controls the cell shape changes that lead to cell separation. The mechanical and regulatory control of this series of shape changes is the topic of this review, in which we synthesize our thinking on the mechanics of cytokinesis with recent discoveries on microtubule-dependent regulation.

Mechanics of cytokinesis

Because the cell is actively re-shaped during cytokinesis, work must be performed. This requires force production and the myosin-II mechanoenzyme plays a major but not exclusive role in this force production [1–3,4•]. These forces act in the mechanical context provided by the viscoelastic cortex. Thus, we have previously proposed a 'balance-of-forces' hypothesis in which the amount of force (F) required to be generated by the contractile ring is counter-balanced by the cortical stretch modulus (S_c), which is the viscoelasticity of the cell's cortex parallel to the plane of the membrane [5•]. The cortical stretch modulus is the energy cost for deforming the cell away from a shape where the surface-area-to-volume ratio is minimized. In this force-balance hypothesis, the initial mother cell may be considered to be at an equilibrium state where the surface-area-to-volume ratio is minimized. As the mother cell passes through the intermediate shapes, the cell moves away from equilibrium. Production of the two nearly spherical daughter cells with minimal surface-area-to-volume ratios represents a return to an equilibrium state. During this process, there is a transition state (energy barrier) where a maximum amount of force is required. As the cortical stretch modulus is the energy cost for deforming the cell, it has a dual role in cytokinesis; prior to the transition state, it acts as a resistor that antagonizes the contractile ring, whereas after the transition state it facilitates furrow thinning as the cell strives to minimize the surface-area-to-volume ratio. Thus, the amount of force that needs to be produced by the contractile ring is proportional to the cortical stretch modulus and the stage of furrow ingression. Yoneda and Dan presented a simple relationship

Figure 1

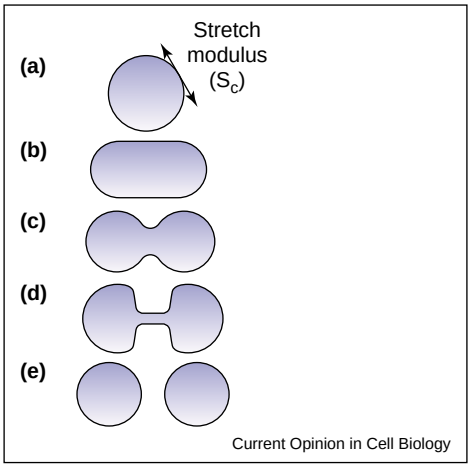
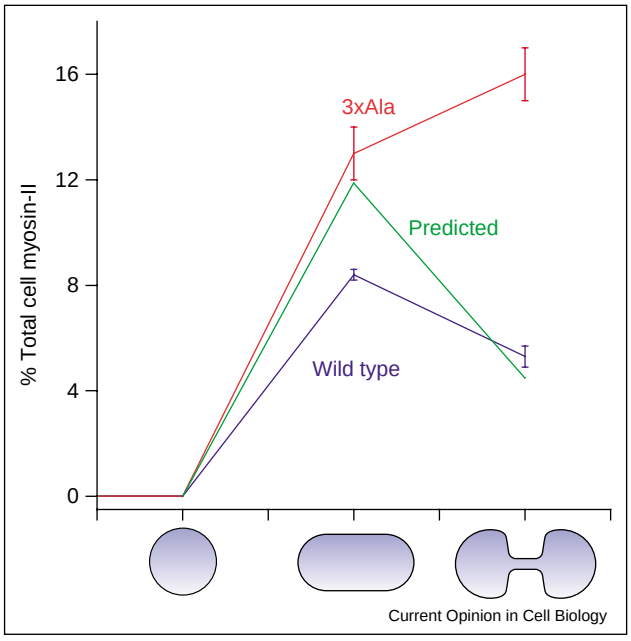


Diagram of the morphological changes of cytokinesis. The highly regimented nature of the shapes make cytokinesis a powerful model of cell shape change that is amenable to physical analyses. **(a)** The cell rounds up into a nearly spherical mother cell. **(b)** The cell elongates forming a cylindrical shape. **(c)** A furrow begins to ingress but maintains continuous curvature with the global cortex. **(d)** A discrete bridge forms that appears as a cylinder connecting the two daughter cells; the curvature of the bridge is discontinuous with the curvature of the two daughter cells. The bridge thins and lengthens. **(e)** The bridge is ultimately severed, producing two daughter cells. These shapes are highly stereotypical, at least for *Dictyostelium*. The cortical stretch modulus, S_c, is the viscoelasticity in the plane of the cortex. It is the energy cost for deforming the cell.

Figure 2



Amounts of wild-type myosin-II recruited to the cleavage furrow cortex follow predicted trends. Actual amounts of *Dictyostelium* myosin-II were measured using fluorescence ratio imaging microscopy. The predicted amounts were determined by considering the minimal force required to maintain cell shape and the biophysical properties and cellular concentration of *Dictyostelium* myosin-II. In contrast, 3xAla myosin-II continues to accumulate in the cleavage furrow cortex, probably because it is not subject to regulated disassembly by myosin heavy chain kinase C.

($F=2R_fS_c\cos\Theta$) where R_f is the radius of the furrow and $\cos\Theta$ is a geometric parameter reflecting the geometry of the cell [6].

Using the Yoneda and Dan relationship, the amounts of myosin-II recruited to the cleavage furrow cortex were accurately predicted for *Dictyostelium discoideum* cytokinesis (Figure 2). First, force requirements were calculated by considering the cortical stretch modulus (1.5 nN/μm), as measured using microcapillary aspiration and the geometry of the dividing cell [7,8]. The myosin-II amounts required in the contractile ring were predicted by converting the force requirements into myosin-II molecules. This conversion was accomplished by considering the amount of force produced per head of myosin-II and the duty ratio (fraction of time spent in a force-generating state per ATPase cycle time). The amounts of myosin-II that are actually recruited to the cleavage furrow cortex were determined empirically using ratio imaging fluorescence microscopy and were found to correspond closely to the predicted values. For example, at the transition state, around 10^5 myosin-II hexameric monomers, representing about 10% of the total cellular myosin-II, were recruited to the cleavage furrow cortex, which is sufficient to produce up to 5 nN of force — extraordinarily close to the 7 nN predicted to be required.

As the cortical stretch modulus is such a potent predictor of the amount of myosin-II that gets recruited to the cleavage furrow cortex, understanding its molecular basis becomes key to understanding the mechanics of cytokinesis. In principle, the cortical stretch modulus is dependent on the cortical viscoelasticity parallel to the plane of the cortex. The cortical viscoelasticity is a material property that reflects the kinetics of actin cross-linking proteins.

Genetic and biophysical studies in *Dictyostelium* have identified proteins involved in cytokinesis that control the cortical stretch modulus and viscoelasticity. Dynacortin and cortexillin are two actin-cross-linking proteins that modulate cortical viscoelasticity and how the cell shape changes with time during cytokinesis [9–11]; Girard *et al.*, unpublished; Zhang and Robinson, unpublished). Dynacortin was identified in a genetic suppression experiment designed to detect suppressors of a *cortexillin-I* mutant [12]. The results of these studies indicate that global actin cross-linking through dynacortin, which is regulated by globally distributed RacE, acts antagonistically to the cleavage furrow cortical mechanics controlled by cortexillin-I and myosin-II.

Actin and myosin-II reorganization during cytokinesis

Actin and myosin-II have long been known to be key players in cytokinesis [1,13,14]. These are highly dynamic cytoskeletal elements, and their dynamics play a critical role in controlling the rates of furrow ingression. The first suggestion that actin dynamics are critical for cytokinesis came from *Drosophila*, in which *twinstar* mutants devoid of cofilin failed at cytokinesis, presumably because they over-accumulated filamentous actin in the cleavage furrow cortex [15]. Subsequently, actin dynamics have been confirmed to play a diverse and complex role in cytokinesis. In *Dictyostelium*, new actin is generated at the poles where Arp2/3 accumulates [16,17]. Rhodamine-labeled actin becomes incorporated into filamentous actin at the poles before flowing towards the cleavage furrow cortex. In mammalian cells, introduction of the barbed-end actin-filament-capping drug, cytochalasin D, into the poles of cells inhibits cytokinesis [18]. When cytochalasin D was introduced into the cleavage furrow region, furrow ingression accelerated. To reconcile these pieces of data, it has been proposed that new actin filaments assemble in the poles, travel to the furrow cortex and are disassembled as the cleavage furrow cortex constricts. As viscosity can be thought of as the energy cost for bulk flow, it is reasonable that removal of actin from the cleavage furrow region would reduce viscosity and cause an acceleration of cleavage furrow ingression [3]. Furthermore, as the contractile ring volume decreases towards the end of cytokinesis, it is expected that the contractile ring has to be disassembled and ultimately removed in order that the intercellular bridge may be severed. In *Schizosaccaromyces pombe*, actin dynamics have been shown to be a critical component regulating the rate of contractile ring constriction [19•,20•]. The assembly and dynamics of the cortical actin in this system depends on Arp2/3, formin, profilin and Wasp. Arp2/3- and formin-mediated nucleation mechanisms represent genetically independent pathways that lead to assembly of the cytokinetic ring in *S. pombe*. Further, both pathways are controlled by cdc15p, a PCH (*pombe* Cdc15 homology) protein [21•]. PCH proteins are known to regulate actin and contractile ring function in organisms ranging from yeasts to mammals [1]. It appears that new actin polymerization, combined with disassembly, regulates furrow ingression constriction [19•]. Interestingly, in *C. elegans*, Arp2/3 is not required for cytokinesis, whereas formin and profilin are, suggesting that formin-mediated nucleation drives actin assembly in this system [22•,23].

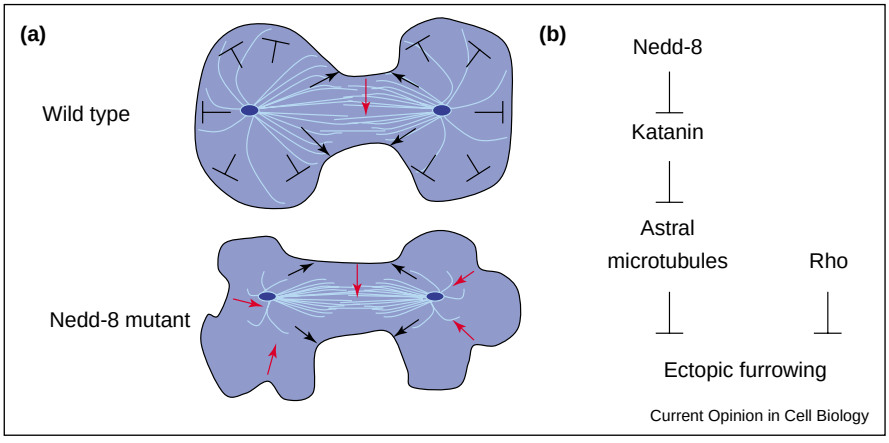
Myosin-II dynamics are also specifically regulated during cytokinesis of *Dictyostelium*. Comparison of the amounts of wild-type myosin-II with those of a mutant myosin-II, 3xAla, that is constitutively assembled into thick filaments indicates that regulation of myosin-II filament assembly/disassembly by heavy chain phosphorylation controls the flux of myosin-II that is sent to the cleavage furrow cortex (see Figure 2). Furthermore, cortical myosin-II turnover dynamics are specifically controlled by myosin-II heavy chain phosphorylation and the entire equatorial myosin-II pool appears to turn over with a half-life of just 7s [24]. Three isoforms of myosin-II heavy chain kinase (MHCK) are found in *Dictyostelium* and one isoform (MHCK C) is recruited to the cleavage furrow cortex late in cytokinesis, presumably to facilitate disassembly of the myosin-II thick filaments for final remodeling of the cortex before daughter cell separation [25•]. Using a novel fluorescence resonance energy transfer (FRET)-based sensor, Chisholm and colleagues show that myosin light chain kinase (MLCK) is globally distributed but that its activity increases in the cleavage furrow of mammalian cells [26•]. Regulatory light chain phosphorylation by MLCK stimulates increased myosin-II activity, although this does not appear to be essential for successful cytokinesis, at least in *Dictyostelium* [27]. The increased MLCK activity in the cleavage furrow cortex may signify a positive feedback mechanism that ensures strong myosin-II contractile activity in the contractile ring.

It is not well understood how myosin-II is targeted to the cleavage furrow cortex of any cell type. In *S. pombe*, a myosin-II progenitor remains stably associated in a punctate spot throughout interphase and the cytokinetic myosin-II ring appears to grow out from this spot [20•,28]. Furthermore, maintenance of the myosin-II spot depends on an UCS (UNC-45/CRO1/SHE4) domain protein, Ring3p. UCS-domain containing proteins facilitate the function of different myosins partly by providing a chaperone function for the motor domain [29•,30].

Regulation from the mitotic spindle during cytokinesis

A pivotal aspect of cytokinesis is the temporal and spatial control of the cortical distribution of actin, myosin and other cortical elements during mitosis and cytokinesis. In the past few years, several elegant studies have further defined how the spindle directs the assembly and contraction of the contractile ring. Several studies over the past decades have implicated either the central spindle or the astral microtubules in guiding the formation of a single central furrow [13]. As different microtubule populations have been implicated in different systems, researchers have suggested that the differences may be due to organism differences. However, in a recent study in *C. elegans*, both astral and central spindle microtubules contribute to defining the contractile zone and the precise geometry of the spindle appears to determine which set of microtubules contributes. Further, it has been suggested that the contractile ring is assembled where the density of microtubules is at a local minimum [31]. This idea is consistent with another recent study in mammalian cells, which suggested that the bipolar spindle is really a combination of two monopolar spindles

Figure 3

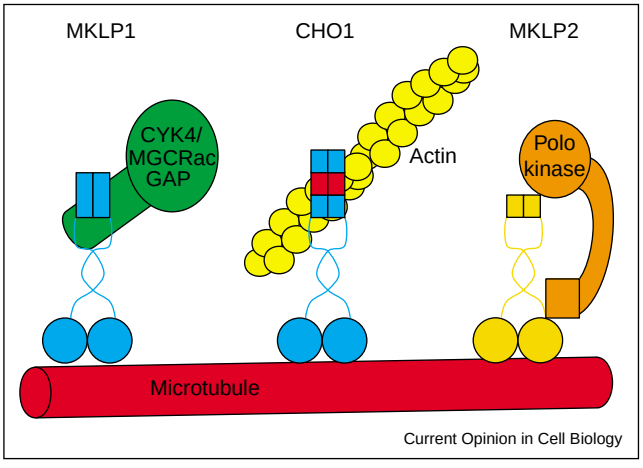


The mitotic spindle provides spatio-temporal control of wild type cytokinesis. **(a)** Schematic relating spindle morphology and furrowing activity of wild type and *Nedd-8* mutants. Red arrows indicate contractile activity. Black arrows represent positive signals that stimulate furrowing. Black inhibition bars indicate inhibitory signals that block ectopic furrow activity. **(b)** Summary of pathway of Nedd-8 control of ectopic furrowing through modulation of katanin and astral microtubules [33•]. Rho also inhibits ectopic furrowing in mammalian cells [34].

[32•]. The microtubules that radiate past the chromosomes become stably associated with the cortex and are proposed to determine the site of contractile ring formation. Perhaps these stable microtubules help stabilize focal-adhesion-like complexes to facilitate the formation of contractile ring actin [1]. By contrast, astral microtubules were observed to be highly dynamic. Highly dynamic microtubules have been proposed to be generated by the microtubule destabilizer katanin. Mutants of katanin in *C. elegans* assemble overly long microtubules, while Nedd8, a ubiquitin-like degradation system, appears to keep katanin activity in check (Figure 3) [33•]. Mutants of Nedd8 fail to inactivate katanin, resulting in short astral microtubules. Intriguingly, Nedd8 mutants display ectopic furrow formation (extra furrows in addition to the normal equatorial cleavage furrow) during cytokinesis, suggesting that the astral microtubules normally keep the local polar cortex quiescent so that the equatorial cortex can contract. Mutations in one of the katanin subunits, encoded by Mei-I, suppresses the *Nedd8* mutant ectopic furrowing activity. The ectopic furrowing activity is reminiscent of the phenotype observed when mammalian cells were treated with Rho inhibitors [34].

Several studies indicate that Rho activation in the region of the cleavage furrow is an essential component of the regulation of new actin polymerization and the timing of cytokinesis. Regulators of Rho including the ECT2/pebble Rho guanine nucleotide exchange factor and the RhoGAP (encoded by *Drosophila* RacGap50C, *C. elegans* cyk-4, and mammalian MgcRacGAP) [35–37] associate with the central spindle. *C. elegans* cyk-4 forms a complex *in vitro* and *in vivo* with the mitotic kinesin-like protein 1 (MKLP1) family member zen-4 (the cyk-4/zen-4 complex is referred to as centralspindlin) (Figure 4) [36]. The RacGAP activity is converted into RhoGAP activity via phosphorylation by Aurora B serine/threonine kinase. The timing of cytokinesis depends on the activity of the anaphase-promoting complex, which controls multiple targets [38]. Some of this regulation is undoubtedly

Figure 4



Mitotic kinesin-like proteins localize to central spindle or midbody microtubules at different stages of cytokinesis. Three protein–protein interactions have been indicated to date. MKLP1 forms complexes with RhoGAPs, Cyk-4 and MgcRacGap. The CHO1 splice variant of MKLP1 includes an extra exon (exon 18), which encodes an actin binding domain. CHO1 can bind filamentous actin. MKLP1s also have a nucleotide-independent microtubule-binding site in their tail domain (not indicated). MKLP2 is phosphorylated by polo-like kinase 1 on multiple sites including a site in the neck linker. Phosphorylation of MKLP2's neck linker site is required for polo-like kinase 1 to localize to the central spindle. The authors propose that polo-like kinase 1 binds the MKLP2 neck linker through its polo box domain for central spindle localization [42•].

mediated by cyclin B and cyclin B3 isoforms, which appear to directly or indirectly regulate Rho activity [39].

MKLP1-family proteins appear to function at several levels during cytokinesis in addition to the role described above for MKLP1 and zen-4. *Drosophila* MKLP1-family protein, pavarotti (Pav-KLP), associates with *polo* kinase in co-immunoprecipitation experiments [40,41]. In mammalian cells, polo-like kinase 1 (plk1) phosphorylates a second MKLP-like protein, MKLP2 (also named rabkinesin6/RAB6-KIFL), and this phosphorylation appears to create a binding site required for the maintenance of plk1 localization to the central spindle (Figure 4) [42•]. By itself, Plk1 weakly binds microtubules, which appear to enhance its activity towards MKLP2. Thus, synergy between MKLP2, plk1 and microtubules helps restrict plk1 to the central spindle, where it presumably phosphorylates additional targets to regulate cytokinesis. CHO1, a splice variant of MKLP1, has an extra domain encoded by a single exon that binds actin (Figure 4). Antibody inhibition studies suggest that CHO1 function through this domain is required for the final severing of the intercellular bridge [43]. Thus, we are beginning to elucidate on a molecular level how the central spindle communicates with the cell cortex.

Conclusions

As well as being a fascinating dynamic cell process in itself, cytokinesis offers a significant repertoire of potential drug target candidates for the treatment of cancer. The pathophysiology of other diseases may also be due at least in part to defects in cytokinesis. For example, brains of patients with the neurological disease globoid cell leukodystrophy develop multinucleated globoid cells, probably as a result of the accumulation of a lipid metabolite, psychosine [44]. A recent study also showed that a cytokinesis defect in mouse cardiac myocytes caused by mutations in nonmuscle myosin-IIB correlates with a novel form of cardiac hypertrophy [45•]. Thus, the development of drugs targeting cytokinesis factors is of considerable medical interest [46,47•].

Cytokinesis is an elegant cell shape change that requires the integrated function of many cytoskeletal systems. The spindle provides spatio-temporal control over the mechanical events of the cell cortex. However, how these signals translate into cortical mechanisms is still not understood. The amount of force generated by the contractile ring must be sufficient to overcome the cortical viscoelasticity of the global cell cortex. It is probable that the ultimate readout from the spindle involves changes in cortical actin cross-linking and myosin-II mechanochemistry, thereby specifying the force-balance that governs the shape changes of cytokinesis. By studying the biochemical and feedback control of the mechanics of cytokinesis, it may be possible to understand how cells complete cytokinesis with such high fidelity in a wide variety of mechanical environments such as in a tissue or blood stream.

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- •• of outstanding interest

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