# *Dictyostelium* cytokinesis: from molecules to mechanics

DOUGLAS N. ROBINSON*, KRISTINE D. GIRARD, EDELYN OCTTAVIANI and ELIZABETH M. REICHL
*Department of Cell Biology, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205, USA*

## Abstract

Cytokinesis is the mechanical process that allows the simplest unit of life, the cell, to divide, propagating itself. To divide, the cell converts chemical energy into mechanical energy to produce force. This process is thought to be active, due in large part to the mechanochemistry of the myosin-II ATPase. The cell's viscoelasticity defines the context and perhaps the magnitude of the forces that are required for cytokinesis. The viscoelasticity may also guide the force-generating apparatus, specifying the cell shape change that results. Genetic, biochemical, and mechanical measurements are providing a quantitative view of how real proteins control this essential life process.

## Introduction

Cells use the mechanical process, cytokinesis, to cleave themselves into two daughter cells (Rappaport, 1996; Robinson and Spudich, 2000b). In this review, we focus on the biochemical basis for this mechanical process considering both the contractile apparatus and the viscoelastic cortex that provides the context for contractility. We will concentrate on research utilizing the cellular slime mold *Dictyostelium discoideum*, a system that has provided numerous insights into the function of the actin cytoskeleton in this dramatic cell shape change. However, we will also consider results from other systems to present a more complete picture for the biochemical bases for the mechanics of cytokinesis.
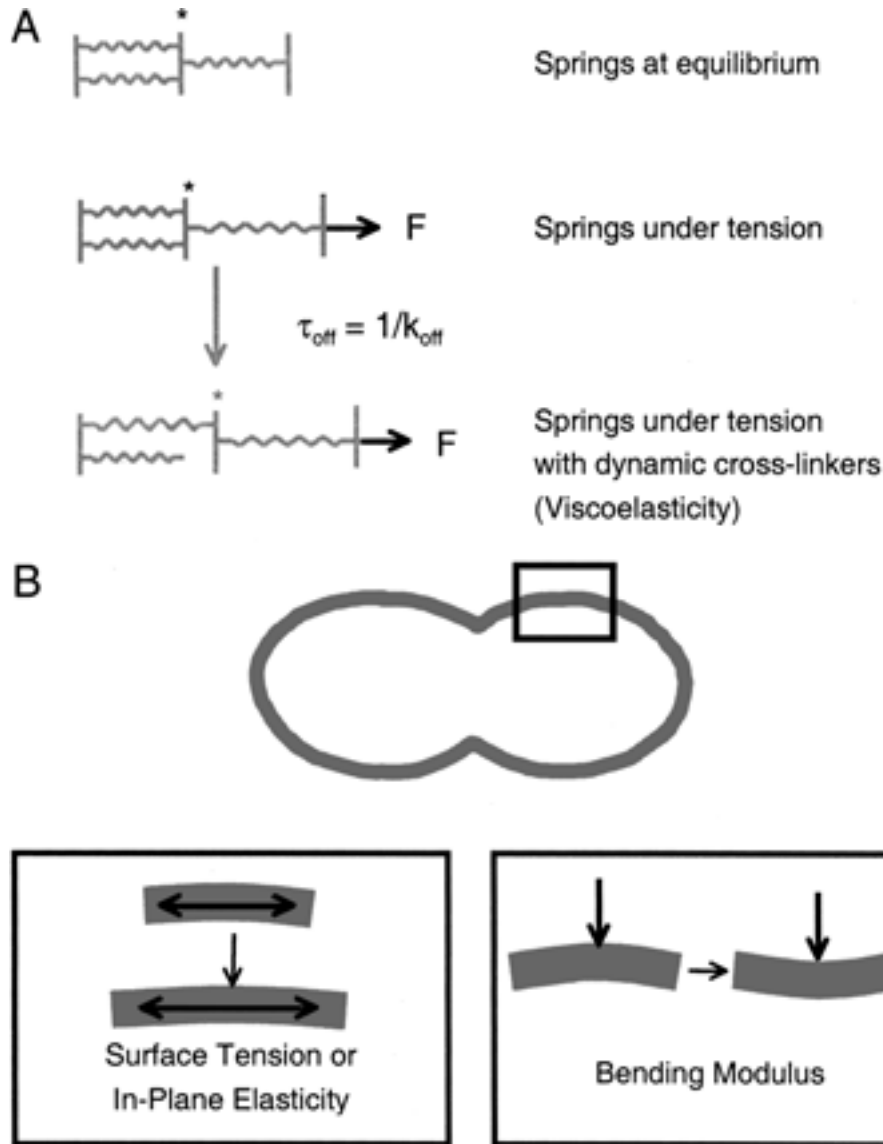
## Mechanical elements of cell shape changes

Mechanical processes, such as cell motility or a cell shape change, must include two major components. One component, force generation, drives the process while a second component is the stiffness and/or viscoelasticity that resists the force (see Howard, 2001). Pictorially, the force elements are denoted as vectors and the stiffness and viscoelasticity are denoted as springs and dashpots (denoted here as springs with connectors characterized by a typical off rate so that $\tau_{off} = 1/k_{off}$) (Figure 1). Because forces can be contractile or expansive, they can push forward the front of a lamellipodium or contract the cleavage-furrow cortex (Rappaport, 1967; Higgs and Pollard, 1999; Welch, 1999). Each type of force must act against the resistive viscoelasticity (Yoneda and Dan, 1972; Matzke *et al.*, 2001). On the other hand, the viscoelastic nature of the cortex also helps to facilitate the shape change by allowing the cortex to assume and maintain new shapes. For example, an expansive lamellipodium may depend on actin cross-linkers to stiffen the polymerizing actin meshwork (Nakamura *et al.*, 2002). Cross-linkers help focus the forces that are advancing the lamellipodium front.

In general, we will refer to the cell as having stiffness. In layman's terms, we intend to imply that the stiffness is the proportionality constant that relates a perturbing force (stress) to the deformation (strain) that it causes. However, the mechanical properties of the cell cortex are far more complex than this and are determined by the viscoelasticity of the cytoskeleton, the cytoskeletal density, and the thickness of the cortex. Further, these mechanical properties differ, depending on the type of stress that is applied (Figure 1). The cortex can be deformed or bent perpendicularly to the plane of the cortex, which reveals the bending modulus. Glass needles and atomic force microscopy predominantly probe the bending modulus (Pasternak *et al.*, 1989; Matzke *et al.*, 2001). Microcapillary aspiration has been applied in several cases to *D. discoideum* and reveals the surface tension or in-plane elasticity (Evans and Yeung, 1989; Gerald *et al.*, 1998; Dai *et al.*, 1999). In another study, the microcapillary aspirator was used to examine how tightly the plasma membrane is attached to the cortical cytoskeleton, revealing an additional physical property of the cell cortex (Merkel *et al.*, 2000). Using reflection interference contrast microscopy, Simson and colleagues monitored how the cell is deformed by laminar flow. From this, they extract the bending modulus, surface tension and adhesion energy (Simson *et al.*, 1998). A summary of the measurements of some of these properties comparing a variety of *D. discoideum*

*To whom correspondence should be addressed: Tel.: +1-410-502-2850; Fax: +1-410-955-4129; E-mail: dnr@jhmi.edu

*Fig. 1.* Mechanical elements define how a cell shape change occurs. A. The major cellular mechanical elements include force production (*F*) and viscoelasticity. A linear system of springs are useful for envisioning how force and stiffness interact to define the mechanical reshaping of a cell. Suppose the goal is to move the asterisk over the vertical bar between two sets of springs. If the springs are at rest then no movement of the asterisk occurs. If tension or force is applied, then the springs will stretch and the amount of movement of the asterisk is determined by the stiffness of the two sets of springs. If the springs are attached to the vertical bar with dynamic cross-linkers, then the springs will have a release rate, $k_{off}$. This off rate will specify the elasticity of the cell, giving the cell a viscoelastic quality. The off rate will not only set the time a cross-linker spends uncross-linked but will also determine the probability that a cross-linker is bound. Collectively, this results in a persistence of cortical elasticity, helping to define different zones with different cortical viscoelastic properties. In the case of the linear springs, after a time period, the cross-linker will release leading to further movement of the asterisk. B. Cell cortices have multiple mechanical properties, of which two are depicted. Surface tension is the tension or elasticity within the plane of the cortex. The bending modulus is revealed when the cortex is bent perpendicularly to the plane of the cortex.

cytokinesis mutants to their respective parental strains is presented in Table 1.

It is useful to remember that cell cortices are not true solids but non-Newtonian fluids. As such, the cell cortex is really a viscoelastic fluid and the nature of the viscoelasticity is specified by the half-life of the actin cross-linkers that interconnect the actin filaments to each other and to the overlying plasma membrane (Wachsstock *et al.*, 1993, 1994; Xu *et al.*, 1998). With this in mind, actin cross-linking proteins and/or regulators of the actin cytoskeleton are antici-

pated to be major determinants of the viscoelasticity of the cell cortex and are anticipated to play important roles in specifying the types of cell shape changes that occur.

From this outline of the physical principles of cell shape changes, the pertinent questions become what are the molecular bases for cleavage-furrow constriction, how is the stiffness or viscoelasticity of the cortex established, and how is local viscoelasticity modulated to dictate the appropriate shape change. We will deal with each of these points in the subsequent sections.

*Table 1.* Summary of proteins and their roles in cortical mechanics

| Protein | Proposed function in cytokinesis | Parental stiffness[#] (%) | Technique | Reference |
|---|---|---|---|---|
| Myosin-II | Equatorial contractility | 70% (bending modulus) | Glass needle | Pasternak *et al.* (1989) |
| | | 30% (surface tension) | Microcapillary aspiration | Dai *et al.* (1999) |
| RacE | Global shape control | 20% (surface tension) | Microcapillary aspiration | Gerald *et al.* (1998) |
| Cortexillin-I, II* | Equatorial stiffness | 70, 100% (surface tension) | Laminar flow | Simson *et al.* (1998) |
| | | 20, 40% (bending modulus) | | |
| Dynacortin | Global shape control | Not measured | | Robinson and Spudich, (2000a) |
| Coronin | Global shape control | Not measured | | deHostos *et al.* (1993) |

[#] Wild type parental stiffness is considered to be 100%.

* The percentages are from single mutants and correspond with cortexillin-I or cortexillin-II, respectively.

## Force-generation drives equatorial constriction

In the cytokinesis field, the source of the force that constricts the cleavage-furrow has been debated (reviewed in Rappaport, 1996). Two extreme classes of simplified models, equatorial constriction and polar relaxation, have evolved. Simply, the equatorial constriction model assigns the source of active force (a contractile force) to the cleavage-furrow cortex, while the polar relaxation model assigns the active force (an expansive force) to the polar cortex. Computer modelling can support either model depending on which assumptions are used (White and Borisy, 1983; Devore *et al.*, 1989). However, some computer programs that model equatorial force generation are better able to predict the outcomes of unusual cleavage events where the cell shape has been altered (Rappaport, 1964; Devore *et al.*, 1989). Early studies using glass needles in echinoderm eggs showed that the cleavage-furrow cortex generated forces, ranging from 20 to 50 nN depending on the species (Rappaport, 1967). In more recent studies, mammalian cells, which were plated onto thin silicon sheets so that their traction forces could be mapped, showed the greatest amount of wrinkling in the cleavage-furrow area. The direction of the wrinkling was parallel to the cleavage plane (Burton and Taylor, 1997). The molecular basis for the cleavage-furrow force generation was suggested when it was discovered that the force-generating, actin-activated myosin-II localized to the cleavage-furrow cortex in a variety of organisms (reviewed in Robinson and Spudich, 2000b). Then, the fortuitous discovery of myosin-II disruption strains and the myosin-II deficiency from antisense experiments in *D. discoideum* provided the first genetic data that myosin-II is required for the process (DeLozanne and Spudich, 1987; Knecht and Loomis, 1987). However, the requirement is conditional since *myosin-II* null cells efficiently divide when provided a surface to which to attach but fail at cytokinesis when the cells are grown in suspension culture (Zang *et al.*, 1997).

The conditional requirement for myosin-II has served to perpetuate the debate about what the force producing mechanism is and where it resides. From this result, three major models have emerged to explain how myosin-II mutants divide if provided an adherable surface. These models are referred to as the equatorial force-generating model (Robinson, 2001), the polar expansion model (Uyeda *et al.*, 2000) and the mechanical lens model (Weber, 2001). We will focus our discussion around the equatorial force-generation model. We direct the reader to these other articles for slightly different views of the mechanics of cytokinesis.

In the equatorial force-generation model, the cleavage-furrow cortex ingresses through the active constriction of the actin contractile ring. The major generator of this force is myosin-II, an actin-activated, force-generating ATPase. This protein converts the energy of ATP hydrolysis to produce work. The amount of work that can be produced is related to the step size of the myosin-II motor domain and the amount of force that it generates (Finer *et al.*, 1994). Further, the amount of force that may be required is determined by the viscoelasticity of the cell cortex (Yoneda and Dan, 1972). Yoneda and Dan (1972) presented a simple equation ($F = 2R_fS_c\cos\Theta$) based on Laplace's Formula that relates the minimal amount of contractile force required to stabilize each intermediate shape as the cell progresses along the cytokinesis pathway to the surface tension (in-plane elasticity) of the cell cortex (Figure 2). For the simple equation to be valid the cytoplasm must be an incompressible fluid and the cell must conserve its volume (Greenspan, 1977). Given these two constraints, the cell must undergo a stereotypical shape change during cytokinesis (Ishizaka, 1966; Greenspan, 1977). This shape change is characterized by the expansion of the polar cortex while the cleavage-furrow cortex ingresses. These two regimes intersect in a region of the cortex forming what are referred to as stationary rings. Stationary rings are observed in dividing cells from a wide variety of organisms including *D. discoideum* (Ishizaka, 1966; Robinson *et al.*, 2002a). The Yoneda and Dan equation allows one to estimate the amount of force required at each stage of cytokinesis by considering only three independent variables (Yoneda and Dan, 1972). These variables are the size of the cell, the surface tension, and the degree of ingression of the cleavage-furrow. The degree of ingression and cell size are manifested in the magnitude of the restoring vector
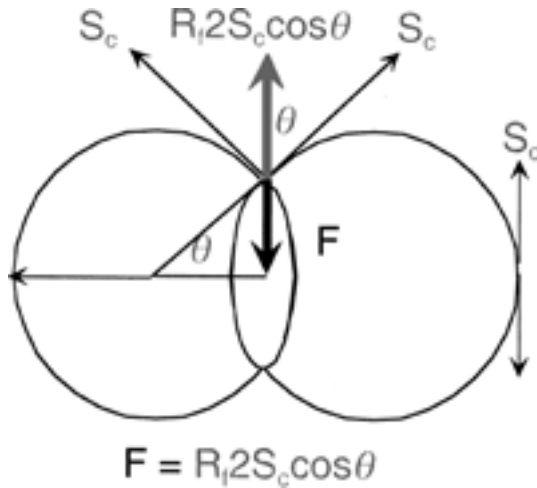
$$F = R_f 2S_c \cos\theta$$

Fig. 2. A simple equation presented by Yoneda and Dan (1972) allows one to predict the minimal amount of force required to stabilize each shape of cytokinesis. The equation relates the amount of force ($F$) to the global surface tension or in-plane elasticity ($S_c$), and the size and degree of ingression of the cell, which are reflected in the furrow radius ($R_f$) and $\cos\theta$.

($2R_f S_c \cos\Theta$) that the force-generating pathway must balance and the radius of curvature ($R_f$) of the cleavage-furrow cortex.

The Yoneda and Dan equation has been applied to a dividing *D. discoideum* cell to estimate the amount of force required at each stage of cytokinesis (Robinson *et al.*, 2002a). From this calculation, the peak amount of required force for an average 10 μm diameter cell is in the range of 6–8 nN. This is similar to the amount of force that has been measured for the much larger echinoderm eggs, which ranged from 20 to 50 nN (Rappaport, 1967). When this calculation is applied to a dividing *D. discoideum* cell, the trajectory of the minimally required force is zero at metaphase when the cell is nearly spherical and is zero when the two nearly spherical daughter cells are separated. This is to be expected since these stages can be thought of as equilibrium states. The minimal required force increases as the cell approaches a cylindrical shape, peaks when the cell is most cylindrical and dissipates as the furrow ingresses further until the daughter cells emerged. The absolute amounts of myosin-II that would be required to generate the amounts of force predicted by the Yoneda and Dan equation were estimated by considering the biophysical properties of myosin-II (Figure 3). This includes the amount of force generated per head of myosin-II per ATP (3.5 pN; Finer *et al.*, 1994), the amount of time a myosin-II head spends strongly attached to the actin per ATP turnover (0.6% for *D. discoideum* myosin-II; Murphy *et al.*, 2001), and the cellular concentration of the protein (3 μM; Clarke and Spudich, 1974, 1977; Robinson *et al.*, 2002a). Ratio imaging fluorescence microscopy was then performed to empirically measure the amount of myosin-II in the cleavage-furrow cortex during the course of cytokinesis (Robinson *et al.*, 2002a). The amount of wild type myosin-II observed was extremely close (within 30%) to
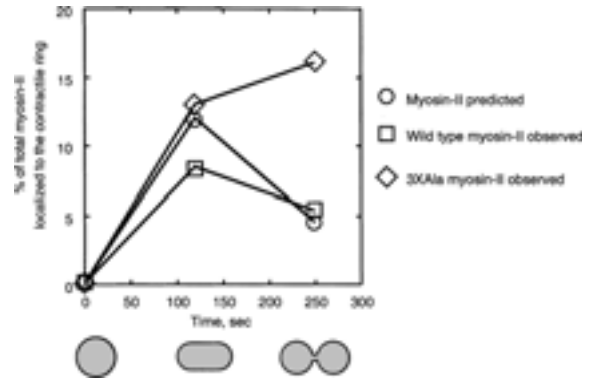


Fig. 3. The amount of myosin-II that is predicted to be required at the cleavage-furrow cortex from simple theoretical considerations is similar to the amount that is actually observed (Robinson *et al.*, 2002a). The percentage of total cellular myosin-II that is predicted (○) to be required at the contractile ring rises as the cell passes from spherical to cylindrical then falls as the cell assumes a dumbbell shape and finally completes division. The percentage of total cellular myosin-II that has been measured (□) at the contractile ring is in close agreement at each stage. A mutant 3 × Ala myosin-II (◇), which escapes phosphorylation control of its assembly state, over-assembles at the cleavage-furrow cortex reaching amounts and concentrations that are significantly greater than observed for wild type myosin-II. The 3 × Ala myosin-II mutant may reflect the actual amounts of myosin-II that pass through the cleavage-furrow cortex during cytokinesis.

the amount predicted at each stage through cytokinesis (Figure 3). In terms of concentration, wild type myosin-II increases by roughly twofold by the end of cytokinesis. The difference in the trends of absolute amounts and concentrations is due to the fact that the cleavage-furrow cortex volume decreases towards zero when the two daughter cells emerge.

In contrast, a mutant myosin-II (3 × Ala) that has had three key threonine residues in the tail of the heavy chain changed to alanine residues overassembles in the cleavage-furrow cortex, reaching a sixfold increase in concentration (Figure 3). The phosphorylation of these threonines, which are targets of heavy chain kinases, regulates the steady state of myosin-II thick filament formation (Egelhoff *et al.*, 1993). The 3xAla myosin-II constitutively assembles into thick filaments and the assembly dynamics are considerably reduced as compared to wild type myosin-II (Sabry *et al.*, 1997; Yumura, 2001). The flux of 3xAla myosin-II may reflect the actual amounts of myosin-II that pass through the contractile ring since it is not subjected to normal disassembly dynamics. Examination of this mutant is important because it shows that the methods used to determine the amounts of myosin-II in the contractile ring are not biased to produce the trends observed for wild type myosin-II. It also provides the first quantitative data showing the consequences of heavy chain phosphorylation in regulating the amounts of myosin-II that are sent to the contractile ring.

Overall, the comparison of the actual amounts of contractile ring myosin-II with the simple theoretical analysis reveals that only three variables (cell size,

degree of cleavage-furrow ingression, and cortical stiffness) are required to reasonably predict how much myosin-II may be required for cytokinesis. Other efforts to mathematically model cytokinesis using principles of fluid mechanics have been presented (He and Dembo, 1997). However, these models do not predict any more accurately the amount of force or myosin-II that is required for cytokinesis. The Yoneda and Dan equation offers simple elegance that is due partly to the relatively few variables needed to predict the amount of myosin-II recruited to the cleavage-furrow cortex. It will be valuable to test the efficacy of this equation to the cytokinesis of other organisms.

The question remains how do *myosin-II* mutant cells divide on surfaces? The answer to this question is still largely unresolved. However, from considering the potential mechanical aspects of cell division, one realizes that significantly less force may be required for the *myosin-II* mutant to divide. Since *myosin-II* mutant cells are only about 30% as stiff in surface tension as compared to their parental counterparts, it should be much easier for these cells to divide, making it possible for residual mechanisms to contribute to the process (Table 1). Traction forces probably play a role, perhaps by helping the cells to elongate into a cylindrical shape, the stage where the greatest force is predicted to be required for cytokinesis. Then, other force-generating mechanisms may produce enough force for final constriction. Other myosin-family members could provide some of this force. For example, members of the brush border myosin-I family have been observed in the contractile rings of dividing mammalian cells (Wagner *et al.*, 1992; Breckler and Burnside, 1994) and multiple myosin-II family members have been implicated in *Schizosaccharomyces pombe* cytokinesis (Bezanilla *et al.*, 1997; May *et al.*, 1997). Another unexplored force-generating mechanism could be the coupling of actin-filament cross-linking with actin-filament disassembly. Indeed, the actin-filament severing protein cofilin is required for cytokinesis in other systems such as *Drosophila melanogaster* where cofilin (*twinstar*) mutants overaccumulate filamentous actin in the contractile rings of dividing spermatocytes (Gunsalus *et al.*, 1995). Consistently, *D. discoideum* cofilin is localized to the contractile ring; however, it appears to be an essential gene, making it difficult to fully evaluate its cellular roles (Aizawa *et al.*, 1995, 1997). In conclusion, additional interaction genetic experiments designed to probe synthetic phenotypes with the *myosin-II* mutants are clearly required to identify the repertoire of relevant force-generating systems that contribute to equatorial contractility of cells.

**The molecular basis for viscoelasticity**

Perhaps the most severe cytokinesis-deficient mutants in *D. discoideum* are the *RacE* mutant cells (Larochelle *et al.*, 1996). The *RacE* mutants have severe defects in cytokinesis when the cells are grown in suspension culture and have an 80% reduction in surface tension (Table 1; Gerald *et al.*, 1998). The RacE small GTPase is evenly distributed around the cell cortex during cytokinesis (Larochelle *et al.*, 1997). Indications of why this mutant might have a softer cortex comes from an analysis of the distributions of two actin cross-linking proteins, dynacortin and coronin (Robinson and Spudich, 2000a). The intensity ratios of dynacortin and coronin between cell surface protrusions and the lateral cortex are about 30% increased in *RacE* mutant cells as compared to their wild type parents. This is most likely due to depletion of these proteins from the lateral cortex. Since the major determinants of cell stiffness are the presence of specific cross-linkers and the concentration of the actin cytoskeleton, this may explain, at least in part, why the *RacE* cells are softer.

A reduction in cell stiffness seems to be the major defect in the *RacE* mutants. Other cortical functions are largely in tact, including the formation of cell surface protrusions, which are likely to be regulated by other Rac-family small GTPases. The *RacE* mutants localize myosin-II to the contractile ring normally and the cleavage-furrows constrict (Larochelle *et al.*, 1996). The cells fail relatively late in cytokinesis and form small blebs, followed by recession of the cleavage-furrow (Gerald *et al.*, 1998). Given the reduction of dynacortin and coronin along the lateral cortex, the blebs may form because the impaired cortex cannot withstand the stress imposed by the mechanical constriction of the cortex. Alternatively, if the cell volume is conserved and the cells are nearly spherical, then roughly 26% additional cortex must be constructed to form the two daughter cells (Robinson, 2001). The *RacE* mutants may be slow at recruiting this additional cortex. The *RacE* phenotype may be revealing an additional activity of the lateral cortex because there appears to be a mechanism that communicates the failure of the lateral cortex to the contracting cleavage-furrow cortex, resulting in cessation of furrow constriction.

The lateral cortex elements, dynacortin and coronin, are likely to be key elements that control the global shape and viscoelasticity of the cell. *In vivo*, both proteins co-localize with filamentous actin and become specifically enriched with dynamic actin structures such as those associated with cell surface protrusions (Maniak *et al.*, 1995; Fukui *et al.*, 1999; Robinson and Spudich, 2000a; Robinson *et al.*, 2002b). *In vitro*, each protein cross-links and bundles actin filaments (deHostos *et al.*, 1991; Robinson *et al.*, 2002b).

*D. discoideum* coronin is the founding member of coronin proteins found in a variety of organisms, ranging from *D. discoideum* to humans (deHostos *et al.*, 1993). Coronin has a WD40-repeat domain at the amino-terminus followed by a coiled-coil, which allows the protein to dimerize (deHostos *et al.*, 1991). The WD40-repeat domain serves as an actin interaction domain and the dimerization through the coiled-coil provides a mechanism for bundling. However, bundling

may not be coronin's only function since coronin from *S. cerevisiae* can nucleate actin polymerization *in vitro* (Goode *et al.*, 1999). Targeted disruption of the *coronin* locus has revealed that coronin is required to maintain normal cell shape (deHostos *et al.*, 1993). The mutant cells become enlarged and multinucleated when grown on surfaces. In suspension culture, a typically more restrictive condition for cytokinesis-deficient growth, *coronin* mutants show a cytokinesis defect that is significantly less severe than the *myosin-II* or *RacE* mutant phenotype. Other proteins such as dynacortin may partially compensate for the loss of coronin (see Figure 4). Consistent with this, *coronin* mutants still form actin-rich surface protrusions to which dynacortin is recruited (Robinson and Spudich, 2000a).

The discovery of dynacortin produced the first genetic evidence that cortical regions interact with each other to produce a cell shape change (Robinson and Spudich, 2000a). Dynacortin was discovered in a genetic experiment designed to identify suppressors of *cortexillin-I* mutants. In this experiment, *cortexillin-I* mutant cells were transformed with an expression cDNA library and the transformed cells were subjected to competitive growth under restrictive conditions for *cortexillin-I* deficient growth. The *dynacortin* cDNA that rescued the *cortexillin-I* mutant encoded only the carboxyl-terminal 181 amino acids (C181). The full-length dynacortin failed to compensate for the loss of cortexillin-I and caused a dominant cytokinesis defect in some genetic backgrounds. C181 also caused a downshift in the Stoke's radius of the endogenous dynacortin, suggesting that it complexes with full-length dynacortin. Indeed, dynacortin purifies from *D. discoideum* as dimers (Robinson *et al.*, 2002b). The primary sequence of dynacortin is unusual because out of its 354 amino

acid residues 100 are serines, threonines and tyrosines. Its $K_d$ for actin binding is in the low μM range, which is physiologically relevant since its cellular concentration is around 2 μM and the concentration of filamentous actin is 70 μM (Haugwitz *et al.*, 1994; Robinson *et al.*, 2002b). From this, greater than 90% of the cell's dynacortin is expected to be bound to actin filaments. However, cell fractionation has suggested that nearly all of the dynacortin is soluble. Since dynacortin is phosphorylated *in vivo*, phosphorylation may be one mechanism of regulating dynacortin's actin association. Intriguingly, dynacortin is distributed along the global cortex in a complementary distribution to cortexillin-I. Dynacortin also appears to be reduced or even excluded from the contractile ring. Thus, C181 might alter dynacortin's actin-filament cross-linking ability, perhaps resulting in global softening and allowing the *cortexillin-I* mutants to divide more efficiently.

Other evidence for global and equatorial pathways that promote cytokinesis comes from analyses of double mutants devoid of both coronin and myosin-II (Nagasaki *et al.*, 2002). These double mutants have phenotypes that are more severe than either single mutant and are especially more defective at cytokinesis on surfaces (*myosin-II* mutants alone have a fully penetrant and severe phenotype in suspension culture). The authors propose that each of these proteins define separate force-generating pathways, a view they described in a recent review (Uyeda *et al.*, 2000). Alternatively, these double mutants may be deficient in separate pathways that control two mechanical elements. The coronin deficiency may disrupt a global shape control pathway that regulates cortical viscoelasticity. Since *myosin-II* mutants have defects in different cortical mechanical properties (Table 1), they may also have quantitative
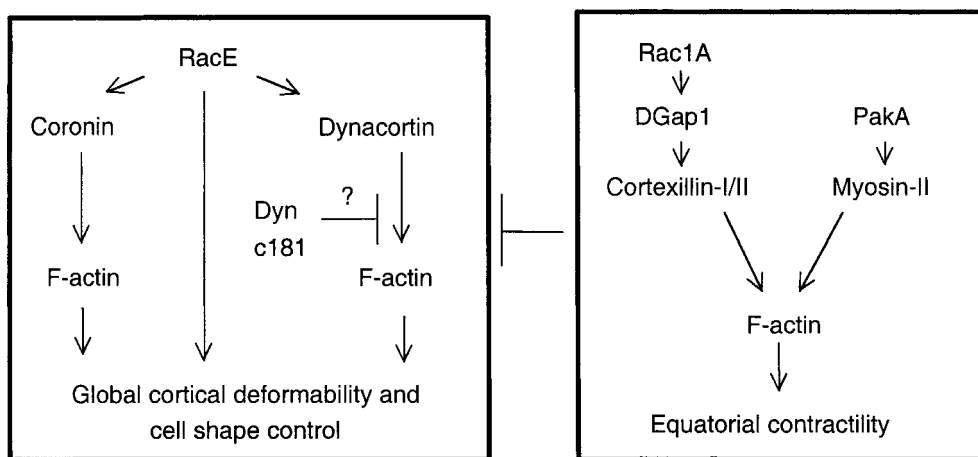


*Fig. 4.* Our current working genetic model describing the circuitry of cytokinesis. In this model, cytokinesis may be described as a dual module system where an equatorial pathway modulates equatorial constriction. Myosin-II is the principal force-generating protein and cortexillins may contribute to the local viscoelasticity. The localization of myosin-II to the cleavage-furrow cortex depends at least in part on the pakA kinase (Chung and Firtel, 1999). The recruitment of the cortexillins to the cleavage-furrow depends on a Rac1 pathway (Faix *et al.*, 2001). The equatorial pathway antagonizes the global cell shape control pathway. The global pathway is controlled by RacE; coronin and dynacortin may be principal actin cross-linking proteins that modulate global surface tension downstream of RacE. Expression of the carboxyl-terminal 181 amino acids (C181) of dynacortin in the presence of endogenous dynacortin suppresses the loss of cortexillin-I. Over-expression of RacE or dynacortin, or the disruption of coronin enhances the *myosin-II* phenotype (Robinson and Spudich, 2000a; Nagasaki *et al.*, 2002), providing further support for two separable pathways that control the global and equatorial cortex so that cytokinesis may proceed.
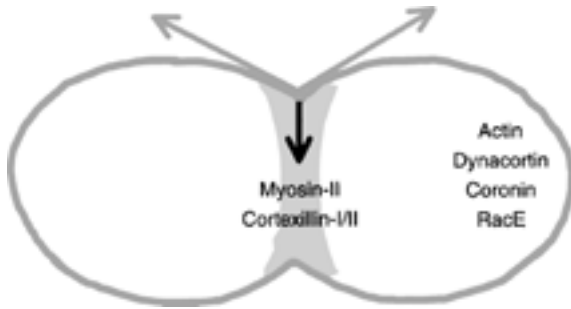
*Fig. 5.* A cartoon depicting the cellular distributions of the current known principal players of cytokinesis. Myosin-II and cortexillin-I/II are enriched in the cleavage-furrow cortex, while RacE, coronin, dynacortin, and actin are globally distributed. While these proteins are enriched in these complementary fashions, they are not restricted to these respective regions. For example, a significant population of myosin-II is localized to the polar cortex during cytokinesis (Robinson *et al.*, 2002a).

defects in global stiffness (surface tension and bending modulus) during cytokinesis. Further, only a small amount of myosin-II actually moves to the cleavage-furrow cortex, while a significant amount remains in the polar (global) cortex (Robinson *et al.*, 2002a). Therefore, the double deficiency of coronin and myosin-II may result in greater impairment of cell shape control, which when further combined with a defect in equatorial contractility leads to a more severe cytokinesis deficiency (Figure 5).

### Regionalization of cortical viscoelasticity

The formation of cortical subdomains that differ in stiffness or viscoelasticity is likely to be important for the resulting cell shape change. Using atomic force microscopy, evidence for stiffness subdomains has been revealed for dividing mammalian cells (Matzke *et al.*, 2001). Because the atomic force microscope indents the cell cortex, the resulting measurement is largely the bending modulus of elasticity. The stiffness (bending modulus) of the whole cell cortex increased slightly by metaphase, and by anaphase the cleavage-furrow region increased in stiffness above the level of the global cortex. The cleavage-furrow stiffness continued to increase throughout cytokinesis, reaching levels 20–30 fold above the stiffness of the global cortex. The progressive increase in cleavage-furrow stiffness is expected since the contractile-ring components are concentrated as the cleavage-furrow cortex volume decreases. However, a stiffness increase by itself does not necessarily reflect the amount of force that is required since it is expected to decrease to zero as the new ground state is reached in the new daughter cells. Indeed, as the contractile ring volume decreased to the end of cytokinesis, the concentration of myosin-II increased while the total amount of myosin-II decreased (Robinson *et al.*, 2002a).

It is anticipated that a local increase in stiffness in the contractile ring is a universal property of cells that exhibit canonical, metazoan-like cytokinesis. Molecu-

larly, the recruitment of specific actin cross-linking proteins is likely to account for this local increase in stiffness. In *D. discoideum*, specific actin cross-linking proteins such as the 30 kDa actin bundling protein and the cortexillins are recruited to the contractile ring (Furukawa and Fechheimer, 1994; Weber *et al.*, 1999). The cortexillins have a spectrin/α-actinin-type actin-binding domain at the amino-terminus followed by a coiled-coil domain and end in a novel 92 amino acid residue domain (Faix *et al.*, 1996). Ordinarily, one might expect the amino terminal actin-binding domain to be required for cortexillin function. However, the carboxyl-terminal 92 amino acids of cortexillin-I are necessary and sufficient for cortexillin-I function in cytokinesis (Weber *et al.*, 1999). *In vitro*, this domain loosely bundles actin filaments (Stock *et al.*, 1999). A nine-residue, lysine-rich region at the carboxyl-terminus is essential for cortexillin-I function and forms one actin-binding site. This region also interacts with phosphatidylinositol 4,5 bisphosphate ($PIP_2$), which suppresses cortexillin-I's bundling activity (Stock *et al.*, 1999). Thus, it is possible that $PIP_2$ regulates cortexillin-I's activity. Cortexillin-II does not seem to share this positively charged stretch so it may be unlikely to interact with $PIP_2$ in this way. Cortexillin-I and cortexillin-II can be co-purified, indicating that they interact directly or indirectly, so perhaps $PIP_2$ may regulate the complex through cortexillin-I (Faix *et al.*, 2001). Intriguingly, the cortexillins associate with Rac1 in pull-down assays using GST-fusion proteins through two Rac1A binding proteins, DGap1 (Faix and Dittrich, 1996) and GapA (Adachi *et al.*, 1997), members of the IQGAP family of proteins (Faix *et al.*, 2001). Elimination of the two IQGAPs from the lysate prevents the cortexillins from associating with Rac1A. The association of cortexillins with GapA, however, only appears after DGap1 has been deleted, suggesting that DGap1 is the principal connector between Rac1A and the cortexillins. Individual deletion of the *cortexillin* genes leads to mild defects in cytokinesis, while the double *cortexillin-I/II* mutants have phenotypes that are significantly more severe. Interestingly, the *cortexillin* mutants show significant defects when the cells are grown on surfaces and in suspension culture. Consistently, elimination of both GapA and DGap1 causes defects in cytokinesis similar to the defects observed in the *cortexillin-I/II* double mutants, suggesting that regulation of the cortexillins through the IQGAPs is essential for normal cytokinesis. In contrast to RacE being a regulator of global cell shape control, Rac1A appears to be a principal regulator of the formation of the cleavage-furrow cortex (Figure 4).

The cortexillins have been shown to have roles in the mechanical properties of cell cortices (Simson *et al.*, 1998). By monitoring changes in cell surface curvature while applying stress to the cell using laminar flow, the bending modulus, surface tension and adhesion energy were calculated. This revealed that the *cortexillin* mutants were reduced to varying degrees in each of these parameters as compared to the wild type parental strain

(Table 1). The cortexillins may increase local viscoelasticity, which will help focus myosin-II contractility. Indeed, the *cortexillin* mutants constrict their contractile rings; however, the contractile zone often appears widened (Weber *et al.*, 2000). In approximately one third of the cytokinesis events, the cleavage-furrow 'slips' towards one end of the cell, resulting in unequal sized daughters. In another approximately one third of the cytokinesis events, the cleavage-furrow 'slips' off the end of the cell, failing to produce daughter cells. Intriguingly, these phenotypes are consistent with what is expected if a spherical mother cell attempted to divide using asymmetrical force without creating asymmetrical stiffness. The path of least resistance would be for the cleavage-furrow cortex to 'slip' off of the end of the cell. An interesting corollary is that if the cell could not produce asymmetrical force, then the whole cortex would simply increase in isometric tension with no invagination of the cleavage-furrow.

## Conclusion

Cytokinesis is the essential mechanical process that results in the division and therefore propagation of the simplest unit of life. It serves as an elegant display for how cells convert chemical energy into mechanical force to accomplish essential tasks. Cytokinesis involves the active constriction of the cleavage-furrow cortex and largely depends on the myosin-II ATPase. The cell's viscoelasticity may define the context for the contractile ring and probably determines exactly how the cortex is reshaped during division. Viscoelasticity likely determines the minimal amount of force that is required to ultimately reshape the cell and helps to focus reshaping so that the cell constricts in the desired manner, typically through the centre or equator of the cell. Uncovering the biochemical bases for the mechanics of cytokinesis and connecting them quantitatively to the two major mechanical elements (force generation and viscoelasticity) will remain a significant challenge. A complete understanding will require the combination of genetics, cell imaging, biochemical studies of purified systems as well as whole cells, and direct cell mechanical measurements of wild type and genetically modified cells. Many of the tools and insights are bringing within reach a comprehensive model of how cytokinesis really works.

## Acknowledgements

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