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Review

Regulation of cytokinesis

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Abstract. At the end of mitosis, daughter cells are separated from each other by cytokinesis. This process involves equal partitioning and segregation of cytoplasm between the two cells. Despite years of study, the mechanism driving cytokinesis in animal cells is not fully understood. Actin and myosin are major components of the contractile ring, the structure at the equator between the dividing cells that provides the force necessary to constrict the cytoplasm. Despite this, there are also tantalizing results suggesting that cytokinesis can occur in the absence of myosin. It is unclear what the roles are of the few other contractile ring components identified to date. While it has been difficult to identify important proteins involved in cytokinesis, it has been even more challenging to pinpoint the regulatory mechanisms that govern this vital process. Cytokinesis must be precisely controlled both spatially and temporally; potential regulators of these parameters are just beginning to be identified. This review discusses the recent progress in our understanding of cytokinesis in animal cells and the mechanisms that may regulate it.

Key words. Cytokinesis; contractile ring; myosin; microtubules; Rho.

Introduction

Cytokinesis is the critical final step of cell division. It is responsible for equal partitioning and separation of the cytoplasm between daughter cells to complete mitosis. In order to generate two identical daughter cells from mitosis, the fidelity of cytokinesis must be precisely controlled, in both establishing the spatial orientation of the cleavage plane and the actual timing of cleavage onset. Unless these restrictions are executed properly, any previous steps taken to ensure fidelity during mitosis are rendered inconsequential. We know from a variety of studies that entry into mitosis is tightly regulated via cell cycle checkpoints. These checkpoints serve as temporary blockades that prevent a cell from progressing through a given cell cycle stage prematurely. Recent evidence for the existence of a cytokinesis checkpoint in yeast [1] underscores the importance of precise temporal control of cytokinesis. In yeast with mutations abolishing dynein function, improper spindle alignment often occurs, and cytokinesis is postponed until the spindle is properly oriented [1, 2]. Muhua et al. [1] have identified mutations that prevent this delay. These yeast mutants continue into cytokinesis before their nuclei can be properly segregated into the mother and daughter cells – resulting in binucleate cells. The existence of this

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checkpoint illustrates the importance of precise coordination between the timing of cytokinesis and nuclear division.

Cytokinesis in animal cells can be divided into a minimum of four discrete steps. During early anaphase, the future site of cleavage furrow formation is specified. It is this site where the mitotic cell will be cleaved into two daughter cells. There is an ongoing debate whether the cleavage plane is dictated by the mitotic spindle or microtubule asters. No matter which is the key player, signals must somehow be generated to assemble actin filaments into the contractile ring. The assembly of the contractile ring is the second step required for cytokinesis. Following actin, myosin II also assembles at the specified cleavage furrow plane, along with a multitude of additional components. Myosin is thought to bind to actin within the contractile ring and provide the necessary force to constrict the cytoplasm of dividing cells. This contraction results in cleavage furrow constriction, the third major stage of cytokinesis. The final stage of cytokinesis involves membrane fusion to physically separate the two daughter cells. What little is known about membrane fusion has been summarized in a recent review [3].

Despite years of study, a number of questions surrounding the process of cytokinesis remain. How is cytokinesis controlled both temporally and spatially? What proteins play key roles in these regulatory mechanisms? Furthermore, once the control switches are released, how is the process executed?

Major among these questions is the nature of cytokinesis regulation. A number of substantial advances in recent years have identified possible key players in the regulatory process. These include small G proteins, their modulators and their target molecules. Studies using a number of experimental systems have shown that mutations affecting the activity of these proteins lead to cytokinesis defects. Furthermore, recent studies have also addressed the possibility that precise control of myosin's contractile activity, via modulation of the phosphorylation state of one of its subunits, could control the onset of cleavage. In addition to these developments on the regulatory front, recent work has called into question dogmas involving mechanistic aspects of cytokinesis as well. Myosin is widely believed to be essential for cytokinesis. However, new data clearly shows that under certain conditions, myosin is not required. These results suggest that it may be necessary to reevaluate current models of cytokinesis. Similarly, recent findings challenge the notion that spindle microtubules are dispensable for cytokinesis once furrow initiation has occurred. It is also intriguing that many studies have now documented interactions between the microtubule and actin cytoskeleton during cytokinesis. Our goal is to examine these recent developments surrounding cytokinesis in animal cells. For additional thoughts on the state of cytokinesis the reader is directed to several excellent recent reviews [3–6].

Small G proteins as molecular switches in cytokinesis

A successful strategy to study cytokinesis has been to focus on the regulation of the mechanical components responsible for contraction of the cleavage furrow – namely the actomyosin cytoskeleton. Concentration of actin at the position of the cleavage furrow is one of the first signs of cytokinesis. Actin has been postulated to act as a scaffolding onto which the rest of the cytokinesis machinery assembles.

During interphase, the key regulators of the actin network include members of the Ras superfamily of guanosine triphosphate (GTP) binding proteins. Members of the Rho GTP family, namely Rho, Rac and Cdc42, mediate distinct actin-based cytoskeletal changes, despite the extensive cross-talk between all three signaling partners [7]. Rho induces stress fiber formation, and potentiates myosin activity through phosphorylation of the myosin regulatory light chain [8–11]. On the other hand, activated Rac produces membrane ruffling and lamellipod formation, whereas Cdc42 induces filopodia (reviewed in [12]). How these proteins regulate the state of the actin cytoskeleton is currently under intense study. Many downstream targets that may link the GTP-switched signals to the coordination of the cytoskeleton have been identified.

Rho GTPases exist in two interconvertible forms: the active GTP-bound form, and the inactive guanosine disphosphate (GDP)-bound form. This allows these Ras-like proteins to serve binary switch functions. The state of bound guanine nucleotide is regulated by a multitude of factors such as GTPase-activating proteins (GAPs) [13], GDPs dissociation inhibitors (GDIs) and guanine nucleotide exchange factors (GEFs) [14]. GAPs and GDIs inactivate GTPases by facilitating hydrolysis of GTP to GDP or the inhibition of GDP/GTP exchange, respectively. On the other hand, GEFs stimulate the exchange of GDP/GTP, thus activating the GTPase signaling molecules. The involvement of these intricately controlled Rho GTPases in actin rearrangement and the rapidly accumulating knowledge of the signaling cascades they participate in have prompted many groups to examine the roles of Rho GTPases in the regulation of the actomyosin system during cytokinesis.

Kishi et al. [15] provided early clues that Rho is involved in both contractile ring and cleavage furrow formation in *Xenopus* embryo, since the microinjection of either rhoGDI or *Clostridium botulinum* C3 transferase, both potent Rho inhibitors, prevented the formation of the cleavage furrow. These results are consistent

with the findings by Rubin et al. that microinjection of C3 transferase into NIH3T3 fibroblasts produces binucleate cells [16]. RhoGDI is known to affect multiple GTPases as downstream targets. The fact that coinjection of GTPγS-bound (constitutively active) Rho but not Rac rescued the rhoGDI inhibitory effects suggests that active Rho is required for both contractile ring and cleavage furrow formation and that specificity must be conferred by yet to be identified factors. The involvement of Rho in cytokinesis appears to be a universal phenomenon, since microinjection of C3 transferase into furrowing sand dollar eggs also rapidly collapses the contractile ring and causes the furrow to regress [17]. However, since C3 transferase disrupts actin organization in general, it is possible that the inhibitory effects may not be confined to the formation of contractile ring but may result from a more general effect on the actin cytoskeleton.

Rho has been shown to regulate a wide array of downstream targets, from phosphotidylinositol 3-kinase, phospholipase D, phosphotidyl 4-phosphate 5-kinase, protein kinase N, rhophilin and rhotekin to multiple tyrosine kinases and the Rho-associated coiled-coil protein kinases ROCK-I and ROCK-II (discussed in [18]). Since the small GTP binding proteins generate widely ubiquitous signals, there arises a critical question regarding the specificity of this signal. One likely mechanism to produce specificity is targeting the signaling molecules to the site of action. One of the first indications that Rho may be targeted to its site of action was provided by Yamochi et al. [19] using *Saccharomyces cerevisiae*, in which the mammalian Rho homolog, RHO1, localized to clustered cortical actin patches of the growth region. Takaishi et al., using myc-tagged RhoA, showed that RhoA was targeted to the cleavage furrow during cytokinesis in NIH3T3 cells [18]. These results suggest the existence of a targeting mechanism able to transport Rho to the cleavage furrow. It is not known if Rho activity is required to switch on the transport system. Since prior injection of rhoGDI or C3 transferase abrogated Rho localization to the cleavage furrow, RhoGTP appears necessary for translocation. This notion is further supported by the observation that RhoGTPγS restored cleavage furrow function in the presence of rhoGDI [15].

However, somewhat contradictory results have been obtained by Dreschel et al. [20], who observed arrested cytokinesis in *Xenopus* eggs by both active and inactive Rho or Cdc42. Although constitutively GTP-bound forms of Rho were generated differently by these two groups (Kishi et al. used GTPγS-bound Rho, whereas Dreschel et al. used RhoG14V point mutant) [15, 20], it is more likely that other variables may explain the difference. For example, both studies used immunofluorescence to localize the target protein; thus the choice of fixation conditions may have generated artifactual localization [20]. Also, staining *Xenopus* eggs undergoing cell division with either antibody or rhodamine phalloidin provides a snapshot of actin organization but lacks the temporal resolution that would allow monitoring of the dynamics of cytokinesis. Although F-actin is localized to the cortex during normal cytokinesis, excess microinjected actin appears to interfere with furrow ingression, suggesting that the actin level in the cortex must be finely balanced. Too little actin prevents the formation of the contractile ring, and provides too little rigidity for tension generation during furrow ingression. In contrast, too much actin may stiffen the cortex, reducing the efficiency of cleavage furrow contraction. Interestingly, perturbation of Cdc42 activity had no effect on assembly of actin into the cleavage furrow but prevented constriction. These results highlight the potential interplay between two Rho GTPases, both of which affect actin reorganization during interphase, and suggests they play distinct roles in regulating cytokinesis [20].

How Rho and Cdc42 work together to promote the successful assembly of actin into the contractile ring and regulate contraction of the cleavage furrow is presently unknown. Current models are based on how these GTPases mediate the reorganization of the actin cytoskeleton in response to growth factors and agonists during interphase. Since Rho induces stress fiber formation, it is possible that the recruitment of Rho to the cortex may regulate a factor crucial for actin polymerization. The fact that cytochalasin B causes a phenotype comparable to that produced by C3 transferase inhibition of Rho supports this hypothesis [21, 22]. The components of the pathway between Rho and actin have not been identified. However, there is now accumulating evidence that myosin may play a key role in transmitting the Rho signal to the actin cytoskeleton. During interphase, myosin regulatory light-chain phosphorylation, which presumably activates myosin, is required for Rho-induced stress fiber formation in fibroblasts . The colocalization of actin and myosin to the cortex and the contractile ring [23] raises the possibility that myosin may be a target of the Rho signal in bundling and cross-linking actin into the cortical array required for furrow ingression.

Recently, Madaule et al. cloned a splice variant of a Rho target protein, citron, which contained at its N-terminal end a kinase domain homologous to those in Rho-associated kinases, ROCK and ROK [24], hence the name citron kinase. Citron kinase contains PDZ (PSD-95, discs large, Z01) and SH3 binding domains at its C terminus, and a zinc finger and a Rho binding sequence within the C-terminal half of the molecule. It colocalized with Rho to the cleavage furrow, and localized to distinct spots associated with tubulin bundles at the midbody when

transfected into HeLa cells. Further dissection of the molecule showed that the Rho binding domain in citron kinase was required for localization, and that overexpression of C-terminus-truncated mutants, lacking the entire C-terminal half up to or including the Rho binding domain, produced multinucleated cells. It is important to note that citron kinase mutants with C-terminal truncations were capable of producing multinucleated cells, regardless of whether further mutations were introduced into the kinase domain, thus rendering the mutant ''kinase-dead''. The role of the serine/threonine kinase domain therefore is unclear at this point. Cells expressing citron kinase C-terminal truncation mutants did undergo normal cytokinesis after telophase, but failed to form the intracellular bridge, and curiously reversed the contraction of their furrows. Since actin organization appeared normal in these cells, it suggested that citron kinase may not affect the actin filaments. The downstream targets of citron kinase, as well as the nature of its interactions with Rho, remain to be elucidated.

On the other hand, it is less clear how Cdc42 regulate cytokinesis. Consistent with the role of Cdc42 in actin reorganization, it is logical to postulate that Cdc42 also mediates the assembly and functioning of the cleavage furrow. However, since inhibiting Cdc42 did not affect actin localization to the contractile ring yet halted proper ingression of the furrow, downstream targets other than actin may be likely. Although the actin contractile ring appeared normal, the possibility that perturbation of Cdc42 may have led to defects in actin organization elsewhere in the cell or that myosin may fail to function properly to facilitate proper ingression cannot be ruled out. The latter model suggests the involvement of a Cdc42 downstream effector on myosin: p21-activated kinases (PAKs). Members of the PAK family have been implicated in Cdc42-mediated cytoskeletal rearrangement [25–29]. More important, PAKs have also been shown to be involved in myosin activity [30–35]. In fact, microinjection of constitutively active PAK arrested cleavage furrow in early *Xenopus* embryo [36], suggesting a cytostatic role of PAK in preventing cells from undergoing cytokinesis until the right time. This result is consistent with the finding that disrupting Cdc42 (either with GDP- or GTP-bound state) resulted in a cytokinesis defect, probably due to a perturbation of the tightly controlled checkpoint. It remains to be elucidated if and how PAKs fit into the Cdc42 signaling cascade in proper cleavage furrow functioning.

The involvement of an additional Rho family member, RacE, was discovered through a genetic screen in *Dictyostelium* [37]. Using insertional mutagenesis, *Dictyostelium* cells were identified based on their failure to grow in suspension where they became multinucleated. In addition, mutations in another Rho family member, RasG, produced a similar phenotype [38]. Much of the current data about the role of these proteins in cytokinesis has been summarized in two recent reviews [3, 39]. However, more recent characterization of RacE mutants has suggested that RacE may affect cytokinesis by altering the integrity of the cortical actin cytoskeleton [40]. Microscopic analysis of cytokinesis in RacE mutant cells in suspension showed formation of a contractile ring and cleavage furrow. However, instead of completing contraction these mutants formed multiple blebs at the poles, and contraction of the cleavage furrow arrested and eventually reversed! Thus RacE does not appear necessary for assembly of the contractile ring or initial constriction. Instead, RacE may be important for maintaining proper cortical tension. This is similar to the likely role for RasG, which also appears to be required for normal actin organization.

If the Rho family of GTPases carry the critical signals to the cortical region of a cell undergoing cytokinesis, how is that signal converted into reorganization of the cytoskeleton? One potential group of effector molecules contains sequences known as formin homology regions, or FH domains [41]. Proteins with FH domains are thought to play a role in organizing different actin structures in a wide variety of organisms. The most compelling evidence comes from a combination of yeast two hybrid, coimmunoprecipitation, and GST pulldown studies wherein FH proteins from various species have been shown to interact with profilin, a key regulator of actin dynamics (discussed in [42]). Bni1p and Bnr1p, both FH family members, bind Cdc42p and Rho4p in *S. cerevisiae*, suggesting a role for FH proteins in linking the Rho GTPase signals to the cytoskeleton. The involvement of FH proteins in cytokinesis is further confirmed by the observation by Swan et al. [42] that *cyk-1* (for cytokinesis defective) mutants, an FH family member, failed to complete furrow constriction in *Caenorhabditis elegans* despite forming a contractile ring that initiates contraction. CYK-1 protein was detected only at the leading edge of the furrow after substantial ingression had occurred. These results suggest that CYK-1 exerts its effect at a late stage of cytokinesis, similar to RasG and RacE, but its precise localization to the furrow suggests it may play a more specific role in cytokinesis. Whether CYK-1 interacts with Rho family members late in cytokinesis awaits further investigation.

The finding that a yeast homolog of CYK-1 protein, cyk1p, exhibits sequence similarity to the mammalian IQGAP further highlights the interwoven connection of GTPase signals and the cytoskeleton during cytokinesis. IQGAPs, which are able to bind Cdc42 and Rac, represent a novel class of molecules that may link GTP signals and the cytoskeleton. Both IQGAP1 and IQ-

GAP2 contain a domain with sequence homology to the actin binding domain of calponin [43], and IQGAP1 has indeed been shown to interact directly with actin [44]. Interestingly, IQGAPs have been shown to inhibit the GTPase activity of Cdc42, prolonging its time in the GTP-bound state [43]. Lippincott and [23] Li have shown that gene disruption of yeast CYK1 resulted in cytokinesis failure with no discernible effects on other events of cell cycle. Cyk1p had a diffuse distribution throughout most stages of cell cycle but localized, together with myosin II and filamentous actin, to the contractile ring at the mother bud junction following the initiation of anaphase. The requirement of IQGAPs and their colocalization to the contractile ring was further confirmed by the finding that disruption of another IQGAP-related gene, Iqg1, also perturbed the proper segregation of nucleus, and caused cytokinesis defects [45]. The fact that overexpression of Iqg1 caused premature actin ring formation was strongly indicative of its roles in the control of actin organization.

Taken together, these recent reports suggest a close connection between GTPase-mediated signals and the actomyosin system during cytokinesis. The data accumulated from cytoskeletal events in response to various extracellular agonists during interphase and our rapidly growing knowledge that the actomyosin system may be regulated by GTPases will surely complement one another to foster a better understanding of how cells coordinate the intricate cross-talk among various signaling pathways to ultimately affect the functions of the cytoskeleton. One important task that remains is to distinguish generalized effects on the actin cytoskeleton from specific effects on the machinery and regulation of cytokinesis.

Myosin phosphorylation as a key regulatory event in cytokinesis

The force for partitioning the cytoplasm is produced by the contractile ring. This transient structure is formed during anaphase at the site of the cleavage plane, along the cell cortex. Actin assembly is one of the first visible signs of contractile ring formation. Short actin filaments are linked by an unknown mechanism to the plasma membrane, and then to each other. These filaments are arranged such that they form a meshwork along the entire circumference at the equator of the dividing cell. Myosin localizes to the cleavage furrow shortly after actin. Bipolar filaments of myosin are thought to bind to adjacent actin filaments and, when myosin is activated, generate tension along the surface of the plasma membrane. Such local constriction of the membrane results in a progressively tighter 'purse string' which segregates the cytoplasm between the dividing cells. Thus, myosin is not only important as a structural component of the contractile ring, but also generates the force necessary to constrict the cleavage furrow.

However, it is important to note that this model of cytokinesis is likely oversimplified. The purse string analogy suggests that actin filaments are arranged in parallel arrays to form an equatorial band of actin. Yet experiments performed by Fishkind and Wang [46] demonstrated that actin localization during cytokinesis is more complex than a simple equatorial band at the cleavage furrow. They observed that actin filaments become progressively more ordered throughout the cleavage process. Furthermore, many actin filaments are aligned along the length of the dividing cell, perpendicular to those filaments near the equator. Based on these findings the authors proposed an alternate model for force generation. This isotropic contraction model postulates that the less-ordered equatorial network of actin and myosin would produce contraction forces originating from many different directions [4, 46], whereas a purse string would generate forces principally perpendicular to the long axis of the dividing cell.

Despite these studies there remains controversy surrounding the validity of the actomyosin-based contractile ring model of cytokinesis. Neujahr et al. [47] found that myosin was not concentrated in the cleavage furrow of *Dictyostelium* dividing in suspension culture or on a solid substrate. They also observed little actin accumulation within the cleavage furrow. These results raise the possibility that myosin-dependent constriction of the contractile ring may not be the driving force behind cytokinesis under these conditions. In contrast, recent studies have demonstrated an enhanced accumulation of fluorescently tagged myosin [48–50] and actin [51] in the cleavage furrow of live *Dictyostelium*.

Myosin II is composed of two myosin heavy chains (MHCs), two essential light chains (ELCs) and two regulatory light chains (RLCs). Proper myosin function requires all three myosin subunits. The RLC is of particular importance because of its role in regulating myosin activity. Phosphorylation of RLC on certain residues increases myosin activity in smooth muscle and nonmuscle vertebrate cells. These activating phosphorylations occur predominantly at serine-19, although threonine-18 phosphorylation is also seen. The principal kinase responsible for phosphorylating these sites is myosin light-chain kinase (MLCK). There is a large body of in vitro biochemical data supporting the belief that phosphorylation of the RLC at MLCK sites is required for myosin activation. However, there are only two examples where the physiological relevance of these phosphorylation sites has been examined in vivo. In both cases the experimental approach was the same: residues equivalent to MLCK sites in the respective organisms were mutated to unphosphorylatable amino

acids, then the mutant RLCs were expressed in null backgrounds. In *Dictyostelium*, there were no defects in myosin-dependent processes, including cytokinesis, suggesting that phosphorylation of RLC at MLCK sites is not critical for myosin function in this organism [52]. However, cytokinesis was defective in germ line cells of *Drosophila*, suggesting that RLC phosphorylation is required for *Drosophila* oogenesis [53]. It is possible that this disparity reflects different requirements for myosin function during cytokinesis in single cells versus cells in the context of a tissue.

In addition to RLC phosphorylation, the MHC is also phosphorylated. These phosphorylation sites in the tail play a critical role in regulating the assembly state of *Dictyostelium* myosin. Mutation of these phosphorylation sites in the tail leads to myosin that cannot assemble or that disassembles poorly [54]. Mutants unable to assemble fail to complete cytokinesis, suggesting that regulation of the myosin assembly state is also important for cytokinesis. Measurements of cortical tension in *Dictyostelium* mutants suggests that the mechanical properties of cells can be separated into RLC- and MHC-regulated components [55], perhaps reflecting the separate roles for regulation of activity by RLC phosphorylation and assembly state by MHC phosphorylation.

Several labs have observed a cell cycle-dependent pattern of RLC phosphorylation. Given that RLC phosphorylation affects myosin activity, it has been proposed that phosphorylation of RLC at specific sites during the cell cycle could regulate the timing of cytokinesis [5, 56]. Localized phosphorylation of myosin in the contractile ring, at serine-19 on the RLC, could activate myosin's motor function, thus initiating constriction of the cleavage furrow. Such a scenario is supported by two recent reports showing the localization of phosphorylated RLC to the cleavage furrow during cytokinesis [57, 58].

DeBasio et al. [57] engineered a fluorescent biosensor to follow myosin phosphorylated on serine-19 of the RLC in live fibroblasts during cell division. They detected a dramatic increase in RLC phosphorylation at serine-19 during anaphase throughout the cell body. This localization becomes more equatorial during telophase, then concentrates in the cleavage furrow during the latter stages of cytokinesis. These findings are consistent with the hypothesis that RLC phosphorylation at serine-19 is important for myosin-mediated constriction of the cleavage furrow. However, localization of phosphorylated RLC is clearly not restricted exclusively to the narrow band of cell cortex occupied by the contractile ring. Yet in a second study the localization of serine-19-phosphorylated RLC in dividing cells was quite discrete. Immunofluorescence results using an antibody that detects RLCs phosphorylated predominantly at serine-19 showed that the antibody stains the midzone between chromosomes during late anaphase [58]. The timing of this localization seemed to be precisely controlled and preceded cleavage furrow formation. The earlier work of DeBasio et al. [57] also found that RLC phosphorylation at serine-19 preceded furrow constriction. Also consistent with the findings using the fluorescent biosensor [57], Matsumura et al. found that phosphorylated myosin persists at the cleavage furrow throughout cytokinesis [58]. Taken together, these results support the idea that phosphorylation of myosin at serine-19 of RLC could be a signal to initiate the furrow constriction phase of cytokinesis.

If RLC phosphorylation were truly a signal to initiate cleavage furrow constriction, it would be vital to regulate the timing of this phosphorylation event so that cytokinesis could not occur prematurely. One such model has been proposed, and it revolves around a link between the timing of cytokinesis and the key cell cycle regulator maturation-promoting factor (MPF) [5, 56]. In eukaryotes, MPF activity is required for entry into mitosis and meiosis. Two of the components that compose MPF are cdc2 kinase and cyclin B. MPF activity peaks during prophase and metaphase, but at the metaphase-anaphase transition MPF activity is greatly diminished due to cyclin degradation. Cdc2 kinase is one of the kinases that can phosphorylate RLC, but not at either serine-18 or serine-19. It phosphorylates RLC at serine-1, serine-2 and threonine-9 in vitro [56]. Phosphorylation at these sites inhibits myosin activity by inhibiting subsequent phosphorylation at the activating residue, serine-19 [59–61]. Thus, myosin activity can be inhibited or activated depending on which residues of the RLC are phosphorylated.

Based on preliminary reports that RLCs could be phosphorylated by cdc2 kinase, Satterwhite et al. [56] examined whether cyclin-cdc2 kinase could phosphorylate intact myosin. Their in vitro data showed that purified cyclin-p34^{cdc2} phosphorylated RLC on serine-1/2 inhibiting sites. Furthermore, in metaphase but not interphase *Xenopus* egg lysates RLC was phosphorylated on serine-1/2. This activity corresponded biochemically with that of MPF. Thus the model proposed by Satterwhite et al. [56] (reviewed in [5]) contends that cdc2 kinase phosphorylation of RLC at serine-1/2 could inhibit myosin activity, and consequently myosin-dependent contraction of the cleavage furrow, until anaphase onset. Phosphatases would then presumably dephosphorylate serine-1/2 residues, allowing for phosphorylation of RLC at serine-19 and myosin activation.

The finding that RLC is differentially phosphorylated during different stages of mitosis was further supported by experiments examining the state of RLC phosphorylation in cultured mammalian cells. RLC from inter-

phase cells was phosphorylated at serine-19, whereas RLC from mitotic cells was phosphorylated predominantly at serine-1/2, consistent with the results of Satterwhite et al. [56]. Upon release of cells from mitotic arrest, RLC became highly phosphorylated at serine-19, whereas serine-1/2 phosphorylation was reduced. This increased level of RLC phosphorylation was maintained 30 min, then gradually decreased throughout the completion of cytokinesis [62]. The levels of RLC phosphorylation at serine-19 were also found to decrease with time using the fluorescent biosensor [57]. These results suggest that the switching of the RLC phosphorylation sites from serine-1/2 to serine-19 during anaphase might be important for regulating the timing of cytokinesis.

Although it is clear that myosin activation, and thus cytokinesis, could be controlled by phosphorylation of RLC at different sites during the early and late stages of mitosis, it is unclear how good a substrate RLC might be for cdc2 kinase in vivo. Yamakita et al. found that cdc2 kinase purified from mitotic cells had very low levels of kinase activity on isolated RLC and intact myosin [62]. Perhaps other kinases besides cdc2 kinase are responsible for RLC phosphorylation at serine-1/2 during the early stages of mitosis. For example, it is known that protein kinase C can also phosphorylate the same RLC residues. Accordingly, two groups have addressed this issue by examining the effects of a specific cdc2 kinase inhibitor, butyrolactone-I, on RLC kinase activity in sea urchin egg extracts. One group observed that the amount of RLC-phosphorylating kinase activity that was sensitive to butyrolactone-I was small [63], suggesting that cdc2 kinase is unlikely to be a major kinase contributing to RLC phosphorylation. However, in a similar analysis butyrolactone-I inhibited the metaphase kinase activity from sea urchin extracts [64]. This kinase activity present in metaphase extracts was also found to phosphorylate RLC at serine-1/2. These conflicting results make it difficult to assess the physiological relevance of cdc2 kinase phosphorylation of RLC. Further experiments are needed to clarify the identity of the kinase responsible for serine-1/2 phosphorylation in vivo.

Several lines of experimental evidence point to the importance of myosin RLC phosphorylation in regulating the timing of cytokinesis. First, myosin phosphorylated on serine-19 of RLC accumulates near the cleavage plane prior to constriction. RLC phosphorylation at serine-19 peaks just before the cleavage furrow contracts. Furthermore, cell cycle-dependent phosphorylation of RLC on different residues can either inhibit or activate myosin function. However, a number of questions remain. What kinases are responsible for the global phosphorylation of RLC at serine-19 during anaphase and how are they regulated? Is phosphorylation of RLC at serine-19 necessary for cytokinesis? Does cdc2 kinase phosphorylate RLC in vivo as a means of preventing premature cytokinesis? It is likely that RLC phosphorylation, and thus myosin activity, involves a dynamic balance between a number of different kinases and phosphatases. The role of phosphatases in cytokinesis is largely unexplored. Is it possible that they represent an important regulatory element during cytokinesis?

Does cytokinesis really require myosin function?

The necessity of myosin function for cytokinesis has been demonstrated in a number of different experimental systems. However, data chiefly from one system, *Dictyostelium*, have caused some to reevaluate not only conventional myosin's role in cytokinesis but also the mechanistic models of cytokinesis itself. Might myosin be dispensable for cytokinesis? Thus, in a classic bit of irony, the system that perhaps produced the most convincing data that myosin was absolutely required for cytokinesis has now required reevaluation of this idea.

The finding that *Dictyostelium* cells deficient in expression of MHCs become severely multinucleate when grown in suspension culture [65, 66] was solid proof that myosin was necessary for cytokinesis. However, these same lines were capable of dividing when maintained on a solid substrate, despite the absence of myosin function. *Dictyostelium* deficient in other myosin subunits, ELCs [67, 68], and RLCs [69], exhibit characteristics similar to the MHC-deficient cells. At the time, the ability of the MHC-deficient cells to divide on a solid substrate was attributed to a cell cycle-independent process called "traction-mediated cytofission" [70]. This phenomenon can best be described as different sections of a cell pulling in opposing directions, producing smaller cell fragments with reduced nuclear content. This process requires adhesive forces between the cell and its substratum.

Recently, Neujahr et al. [71] reexamined the ability of *Dictyostelium* MHC-null cells to divide on a solid substrate. By staining cells for DNA and tubulin, they determined that many of the cells maintained on a solid substrate divided their cytoplasm in a cell cycle-dependent fashion despite the absence of myosin. In addition, mononucleate MHC-null cells proceeded through division on solid substrate with a speed similar to that of wild-type cells. This suggests that although the presence of myosin might make cell division on solid substrate more efficient, cells are clearly capable of dividing without it. Why would myosin be required for cytokinesis under one condition, but not another? Do all cells have a myosin-independent cleavage mechanism?

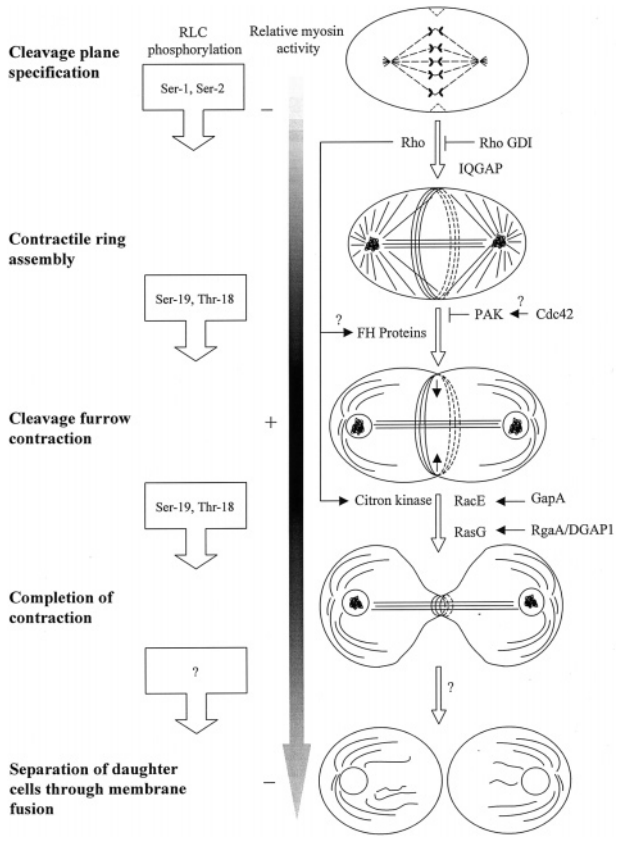

Figure 1. The key participants and regulatory points of cytokinesis. It is certain that this diagram is overly simplified.

A complementary study addressed this perplexing issue by examining the *Dictyostelium* MHC-null cells trying to divide in the absence of an adhesive surface. MHC-null cells were suspended in a droplet or placed on a hydrophobic surface to reduce cell substrate adhesion and imaged over time. Following mitosis, cells remained round, never formed a cleavage furrow and failed to divide [49]. This suggests that myosin is the key force-generating molecule during division in suspension, and that in its absence no other molecules are able to produce the same level of force necessary for contraction. In the light of these data and the finding that MHC-null cells can divide on a solid substrate, the authors proposed that cell division can occur via two different mechanisms – one that is myosin-dependent and one that is myosin-independent but requires cell adhesion. These results, as well as those from Neujahr et al. [71], and myosin subunit gene knockouts [65, 66, 68, 69] suggest that while myosin's presence can facilitate substrate-dependent cleavage, it is not required. Perhaps the additive effects of adhesive forces plus forces generated by some other molecule are sufficient for cytokinesis on a solid substrate. What might these other force-generating molecules be? It is possible that any of a number of different unconventional myosins could fill the role. Alternatively, perhaps all that is needed is elevated cortical tension such as might be provided by increased actin cross-linking [72].

To begin dissecting out important factors influencing substrate-dependent cleavage, Neujahr et al. [73] examined multinucleate MHC-null cells to address the contributions of microtubules to cleavage furrow formation. Adherent multinucleate MHC-null cells (previously grown in suspension) were shown to undergo cell division in a cell cycle-dependent manner using GFP-α-tubulin to follow microtubules in multinucleate cells undergoing cell division. In addition to having mitotic spindles, the observation that cells fail to exhibit extensive leading edge movement suggests they were not separating via migration of the cells away from each other. Taking the results of both papers together [71, 72], it is clear that both mono- and multi-nucleate *Dictyostelium* devoid of myosin function can undergo cell division in a cell cycle-regulated manner.

All of these analyses were performed using *Dictyostelium*, a simple eukaryotic organism. The question remains how applicable these results might be to other cells. Is there a difference in the cleavage mechanisms used by single cells and cells in the context of a tissue? Could mammalian cell division on a solid substrate be affected by traction forces in a similar manner? A novel experimental design, using an elastic substrate, has been employed to measure the traction forces generated by animal cells during cytokinesis [74]. Traction force was detected at the cleavage furrow during cytokinesis. This force increased as daughter cells spread and dissociated. These experiments suggest that tension can promote stretching and separation of the cytoplasmic bridge during cytokinesis. Given these findings, it seems important to consider how this adhesion component could influence cytokinesis in adherent cells.

Taken together, these recent findings provide strong support for the concept that cytokinesis in suspension is likely to be mechanistically different from that of single cells performing cytokinesis on a solid substrate. It is clear that in the case of *Dictyostelium*, myosin is absolutely required for cytokinesis in suspension, but is dispensable for cell division on a solid substrate. Perhaps the two processes involve different key players. Furthermore, cytokinesis within the context of a complex tissue may be different than either mechanism of division for isolated single cells. Any models of cytokinesis that involve adhesion, whether on a solid substrate or within a complex tissue, need to evaluate the influence of this traction component.

Interplay between microtubule and actin networks during cytokinesis

Mitosis clearly requires precisely regulated reorganization of both the microtubule and microfilament cytoskeletal systems. It is well established that microtubules provide the signals necessary for specification of the cleavage plane during cytokinesis. It has been proposed that interaction between the microtubule and actin cytoskeleton systems during cytokinesis could serve to position the cleavage furrow and initiate ingression [75–77]. In addition, the work discussed below suggests that microtubules may play an important role in cleavage furrow contraction. This suggests that microtubules and the actomyosin contractile ring might be intimately linked during these stages of cytokinesis.

Several recent studies by Wang and colleagues have examined the role of spindle microtubules during cytokinesis in cultured animal cells. Nocodazole treatment of cells to reduce the number of midzone microtubules in proximity to the cortex resulted in furrow inhibition or regression [78]. This supports the notion that midzone microtubules are necessary throughout cleavage furrow ingression in cultured cells. These findings are inconsistent with the earlier belief that microtubules were only required for cleavage furrow specification, and that they were unnecessary for cytokinesis beyond that point.

Analysis of cleavage furrow formation in multinucleate *Dictyostelium* myosin heavy chain mutants expressing GFP tubulin suggests that interaction between astral microtubules and the cell cortex exerts a local influence on the cortex [73]. Interaction of microtubules with the cell cortex stimulated ruffle formation and consequently suppression of furrow formation in areas of active ruffling. Since the density of astral microtubules, and therefore ruffling, was lowest between the adjacent asters, furrows formed in these regions – despite the absence of myosin. Since these furrows contracted, continued presence of microtubules is not necessary to complete contraction. These observations suggest that, in *Dictyostelium*, microtubules play an important role in initiation of furrow contraction, but not necessarily progression.

The idea that cleavage furrows may form between sites of astral microtubule-stimulated cortical activity has also been supported by computer simulations [79]. These simulations suggest a distance dependence of the ability of microtubules to stimulate the cortex. Based on geometrical considerations this modeling suggests that beyond a threshold distance the ability to stimulate the cortex would drop off rapidly. Such a model would make it unlikely that astral microtubules would stimulate contraction in the equatorial region.

The involvement of microtubules in furrow formation has been addressed in other experimental systems as well. The drug colchicine, which binds to tubulin monomers and causes microtubule depolymerization, was applied to different regions of dividing newt eggs. Furrow formation stimulated by treatment of newt eggs with furrow-inducing agents was ablated when colchicine was applied locally to the furrow [80]. Furthermore, local colchicine application shortly following furrow constriction resulted in arrest and, sometimes, retraction of the furrow. These findings suggest that in newt eggs, microtubules are required for both furrow formation and contraction.

Further evidence underscoring the importance of midzone microtubules for progression through cytokinesis comes from *Caenorhabditis elegans* embryos, where null mutants for a kinesin-like protein, ZEN-4, result in multinucleate embryos due to premature arrest of the cleavage furrow [81]. ZEN-4 is a member of the MKLP1 family of proteins that promote bundling of antiparallel microtubules. Consistent with the loss of this bundling activity, the midzone microtubules of ZEN-4 mutants are dissociated. Failure to complete contraction of the cleavage furrow demonstrates the importance of intact midzone microtubules for progression through cytokinesis in *C. elegans* embryos. This contrasts with the results of Neujahr et al. [73] in *Dictyostelium*, where microtubules were not necessary for completion of furrow contraction. Perhaps different organisms have different requirements for continued furrow ingression.

Fishkind et al. [82] have examined the role of microtubules in actin organization in cultured mammalian cells by treating dividing cells with drugs that either disrupt or stabilize spindle microtubules. Following nocodazole treatment to disrupt microtubules during anaphase, cells exhibited generally less organized actin localization patterns than untreated cells, although equatorial bands of actin filaments were unaffected. In contrast, when cells were treated with taxol to stabilize microtubules, actin filaments were highly ordered along the equator of the dorsal and ventral planes. These experiments suggest that spindle microtubules may influence actin organization in the contractile ring, perhaps in part by affecting cortical flow of actin filaments to and from the equator.

However, it is still not clear whether microtubules stimulate or inhibit cortical flow of actomyosin. Cortical flow of actin filaments along the cell cortex is thought to be important for a number of cell movements, including cytokinesis [77]. To investigate cortical flow, Canman and Bement [83] developed an inducible *Xenopus* oocyte system that overcomes some of the difficulties in studying the effect of microtubules on actomyosin-based cortical motility in live cells. Wang and colleagues [82] have shown that cortical flow is confined to the furrow region during cytokinesis. Canman and Bement found that increased microtubule polymerization inhibited cortical actin assembly during cortical flow, whereas microtubule depolymerization increased cortical flow. These results

imply that microtubules, or their associated proteins, might inhibit cortical flow of actomyosin. How might this occur? The authors favor a model whereby microtubule dynamics might modulate the actomyosin cytoskeleton biochemically. This model is supported by data showing that depolymerization of microtubules altered myosin localization [84], and regional disassembly of actin networks was associated with microtubules [85].

Further evidence of the influence of microtubules on actomyosin during cytokinesis comes from additional experiments from Wang et al. [86]. They used a topoisomerase inhibitor to prevent chromosome separation to test whether signals transported via interzone microtubules provoke cytokinesis. Treatment of dividing cells with the topoisomerase inhibitor prevented normal interzone spindle formation. Furthermore, actomyosin failed to form an equatorial band in furrowing cells, but instead localized only to the lateral edges of the cleavage furrow. Accordingly, anomalies in cytokinesis were characterized by distorted or misplaced cleavage furrows, or premature furrow regression. These results suggest that aberrant organization of the microtubule network during cytokinesis affects proper assembly of actomyosin into a contractile ring.

Recent studies of *Drosophila* profilin mutant, chickadee, have addressed the connection between the central spindle and the contractile ring [87]. Analysis of male meiosis in these mutants showed that they exhibited severe cytokinesis defects. Surprisingly, profilin mutations prevented both central spindle and contractile ring formation. To test whether there was cooperativity between spindle microtubules and the contractile ring, they looked at other mutations or drugs that disrupt either cytoskeletal system for similar effects on meiosis. Simultaneous disruption of the central spindle and the contractile ring was observed in cytokinesis-defective mutants of diaphanous, a formin family member, and KLP3A, a kinesin-like protein. Cytochalasin B-treated meiotic cells exhibited the same phenomena. These results strongly suggest that there is an interdependence of the central spindle and the contractile ring during cytokinesis. Even more surprising is the possibility that defects in assembly of the actin-based contractile ring affect spindle assembly.

Further studies elucidating the function of microtubules during cleavage furrow initiation and progression will likely center on the mechanisms for this influence. Given the recent data indicating that (i) midzone microtubules are required for continued furrow ingression in some organisms and (ii) these microtubules can affect contractile ring assembly, it will be important to determine whether the continued presence of spindle microtubules is required for maintenance of the contractile ring throughout cytokinesis. Another future area of study derives from the finding that defects in the actin contractile ring formation disrupt the mitotic spindle. What is the nature of this cross-talk between these two cytoskeletal networks?

Future perspectives

Much is still unknown about how cytokinesis is precisely coordinated during mitosis. Certainly the search for the regulatory pathways involved will continue to be a major focus of research activity for years to come. Faced with the daunting task of identifying potential key regulatory proteins among the thousands present within a cell, it is encouraging that new candidates have been identified. These recent studies demonstrate that members of the small G protein family, their modulators and their effector molecules play important roles in cytokinesis. Further experiments are necessary to delineate precisely those roles. Do they simply serve to properly organize actomyosin into the contractile ring at the right time or are they important for other aspects as well? Another important line of investigation into possible mechanisms of regulating the timing of cytokinesis is the role of myosin RLC phosphorylation. While there is clearly much data to support the proposed model [5, 56], the answer to the most critical question remains unclear. Is phosphorylation of RLC at serine-19 required for cytokinesis? Until this question is conclusively answered in a mammalian system, the model will remain speculative.

In addition to these developments surrounding regulatory aspects of cytokinesis, other recent work has raised questions about widely held mechanistic elements of cytokinesis. Myosin is thought to be essential for cytokinesis in a number of organisms. However, recent results suggest that myosin might not be necessary under certain growth conditions. In at least one organism, *Dictyostelium*, cells can undergo cytokinesis just fine without myosin. It seems that in this case, the sole requirement for these cells to complete division is adhesion between the cell and a solid substrate. While it is possible that this is merely a phenomenon limited to *Dictyostelium*, it seems worth examining in cells from other organisms as well, particularly adherent cultured mammalian cells. Such studies could give significant insight into other key molecules important for cytokinesis.

Moreover, recent studies in some organisms have demonstrated that microtubules can play an important role in continued cell cleavage and are not necessarily dispensable once furrow initiation is completed. The finding that this continual requirement for microtubules is organism-specific comes as no great surprise. While the major tenants of cytokinesis might be applicable to

a wide variety of organisms, it is likely that some variations exist. Nonetheless, it will be worthwhile to determine how common the requirement of microtubules is for the completion of cytokinesis. Lastly, new developments suggest that the mitotic spindle and contractile ring are intimately linked during cytokinesis. The influence of microtubules on the actin cytoskeleton has been studied for quite some time. Although the link has not been completely elucidated, it is clear that some important relationship exists. Indeed, knowledge of the nature of that variation may provide useful insights into the process of cytokinesis. What is more surprising is that defects in the actin contractile ring affect the mitotic spindle. This suggests that the link between these two cytoskeletal elements transmits signals both ways. It will be important to determine which molecules facilitate these interactions as well as how they are controlled. Finally, not only is the regulation of cytokinesis an important and fundamental biological process interesting in its own right, but it may represent an important target for therapeutic interventions in cancer.

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1 Muhua L., Adames N. R., Murphy M. D., Shields C. R. and Cooper J. A. (1998) A cytokinesis checkpoint requiring the yeast homologue of an APC-binding protein. Nature **393:** 487–491
2 Yeh E., Skibbens R. V., Cheng J. W., Salmon E. D. and Bloom K. (1995) Spindle dynamics and cell cycle regulation of dynein in the budding yeast *Saccharomyces cerevisiae*. J. Cell Biol. **130:** 687–700
3 Glotzer M. (1997) The mechanism and control of cytokinesis. Curr. Opin. Cell Biol. **9:** 815–823
4 Fishkind D. J. and Wang Y.-L. (1995) New horizons for cytokinesis. Curr. Opin. Cell Biol. **7:** 23–31
5 Satterwhite L. L. and Pollard T. D. (1992) Cytokinesis. Curr. Opin. Cell Biol. **4:** 43–52
6 Oegema K. and Mitchison T. J. (1997) Rappaport rules: cleavage furrow induction in animal cells. Proc. Natl. Acad. Sci. USA **94:** 4817–4820
7 Hall A. (1998) Rho GTPases and the actin cytoskeleton. Science **279:** 509–514
8 Amano M., Ito M., Kimura K., Fukata Y., Chihara K., Nakano T. et al. (1996) Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). J. Biol. Chem. **271:** 20246–20249
9 Chrzanowska-Wodnicka M. and Burridge K. (1996) Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. J. Cell Biol. **133:** 1403–1415
10 Amano M., Chihara K., Kimura K., Fukata Y., Nakamura N., Matsuura Y. et al. (1997) Formation of actin stress fibers and focal adhesions enhanced by Rhokinase. Science **275:** 1308–1311
11 Kimura K., Ito M., Amano M., Chihara K., Fukata Y., Nakafuku M. et al. (1996) Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science **273:** 245–248
12 Chrzanowska-Wodnicka M. and Burridge K. (1992) Rho, rac and the actin cytoskeleton. Bioessays **14:** 777–778
13 Lamarche N. and Hall A. (1994) GAPs for rho-related GTPases. Trends Genet. **10:** 436–440
14 Narumiya S. (1996) The small GTPase Rho: cellular functions and signal transduction. J. Biochem. (Tokyo) **120:** 215–228
15 Kishi K., Sasaki T., Kuroda S., Itoh T. and Takai Y. (1993) Regulation of cytoplasmic division of *Xenopus* embryo by rho p21 and its inhibitory GDP/GTP exchange protein (rho GDI). J. Cell Biol. **120:** 1187–1195
16 Rubin E. J., Gill D. M., Boquet P. and Popoff M. R. (1988) Functional modification of a 21-kilodalton G protein when ADPribosylated by exoenzyme C3 of *Clostridium botulinum*. Mol. Cell. Biol. **8:** 418–426
17 Mabuchi I., Hamaguchi Y., Fujimoto H., Morii N., Mishima M. and Narumiya S. (1993) A rho-like protein is involved in the organisation of the contractile ring in dividing sand dollar eggs. Zygote **1:** 325–331
18 Takaishi K., Sasaki T., Kameyama T., Tsukita S. and Takai Y. (1995) Translocation of activated Rho from the cytoplasm to membrane ruffling area, cell-cell adhesion sites and cleavage furrows. Oncogene **11:** 39–48
19 Yamochi W., Tanaka K., Nonaka H., Maeda A., Musha T. and Takai Y. (1994) Growth site localization of Rho1 small GTP-binding protein and its involvement in bud formation in *Saccharomyces cerevisiae*. J. Cell Biol. **125:** 1077–1093
20 Drechsel D. N., Hyman A. A., Hall A. and Glotzer M. (1997) A requirement for Rho and Cdc42 during cytokinesis in *Xenopus* embryos. Curr. Biol. **7:** 12–23
21 Bluemink J. G. (1971) Cytokinesis and cytochalasin-induced furrow regression in the first-cleavage zygote of *Xenopus laevis*. Z. Zellforsch. Mikrosk. Anat. **121:** 102–126
22 Luchtel D., Bluemink J. G. and de Laat S. W. (1976) The effect of injected cytochalasin B on filament organization in the cleaving egg of *Xenopus laevis*. J. Ultrastruct. Res. **54:** 406–419
23 Lippincott J. and Li R. (1998) Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis. J. Cell Biol. **140:** 355–366
24 Madaule P., Eda M., Watanabe N., Fujisawa K., Matsuoka T., Bito H. et al. (1998) Role of citron kinase as a target of the small GTPase Rho in cytokinesis. Nature **394:** 491–494
25 Daniels R. H., Hall P. S. and Bokoch G. M. (1998) Membrane targeting of p21-activated kinase 1 (PAK1) induces neurite outgrowth from PC12 cells. EMBO J. **17:** 754–764
26 Dharmawardhane S., Sanders L. C., Martin S. S., Daniels R. H. and Bokoch G. M. (1997) Localization of p21-activated kinase 1 (PAK1) to pinocytic vesicles and cortical actin structures in stimulated cells. J. Cell Biol. **138:** 1265–1278
27 Zhao Z., Manser E., Chen X., Chong C., Leung T. and Lim L. (1998) A conserved negative regulatory region in α-PAK: inhibition of PAK kinases reveals their morphological roles downstream of Cdc42 and Rac1. Mol. Cell. Biol. **18:** 2153–2163
28 Sells M. A., Knaus U. G., Bagrodia S., Ambrose D. M., Bokoch G. M. and Chernoff J. (1997) Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. Curr. Biol. **7:** 202–210
29 Manser E., Huang H. Y., Loo T. H., Chen X. Q., Dong J. M., Leung T. et al. (1997) Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. Mol. Cell. Biol. **17:** 1129–1143
30 Chew T., Masaracchia R. and Wysolmerski R. (1998) Phosphorylation of myosin II regulatory light chain by a member of the p21-activated kinase (PAK) family, γ-PAK. J. Muscle Res. Cell Motil., in press
31 Tuazon P. T., Stull J. T. and Traugh J. A. (1982) Phosphorylation of myosin light chain by protease activated kinase I. Biochem. Biophys. Res. Commun. **108:** 910–917
32 Tuazon P. T., Stull J. T. and Traugh J. A. (1982) Phosphorylation of myosin light chain by a protease-activated kinase from rabbit skeletal muscle. Eur. J. Biochem. **129:** 205–209
33 Tuazon P. T., Spanos W. C., Gump E. L., Monnig C. A. and Traugh J. A. (1997) Determinants for substrate phosphorylation by p21-activated protein kinase (gamma-PAK). Biochemistry **36:** 16059–16064

34 Ramos E., Wysolmerski R. B. and Masaracchia R. A. (1997) Myosin phosphorylation by human cdc42-dependent S6/H4 kinase/gammaPAK from placenta and lymphoid cells. Recept. Signal Transduct. **7:** 99–110

35 Brzeska H., Knaus U. G., Wang Z. Y., Bokoch G. M. and Korn E. D. (1997) p21-activated kinase has substrate specificity similar to *Acanthamoeba* myosin I heavy chain kinase and activates *Acanthamoeba* myosin I. Proc. Natl. Acad. Sci. USA **94:** 1092–1095

36 Rooney R. D., Tuazon P. T., Meek W. E., Carroll E. J., Hagen J. J., Gump E. L. et al. (1996) Cleavage arrest of early frog embryos by the G protein-activated protein kinase PAK I. J. Biol. Chem. **271:** 21498–21504

37 Larochelle D. A., Vithalani K. K. and De Lozanne A. (1997) Role of *Dictyostelium* racE in cytokinesis: mutational analysis and localization studies by use of green fluorescent protein. Mol. Biol. Cell **8:** 935–944

38 Tuxworth R. I., Cheetham J. L., Machesky L. M., Spiegelmann G. B., Weeks G. and Insall R. H. (1997) *Dictyostelium* RasG is required for normal motility and cytokinesis, but not growth. J. Cell Biol. **138:** 605–614

39 Chisholm R. L. (1997) Cytokinesis: a regulatory role for Ras-related proteins? Curr. Biol. **7:** R648–650

40 Gerald N., Dai J., Ting-Beall H. P. and De Lozanne A. (1998) A role for *Dictyostelium* racE in cortical tension and cleavage furrow progression. J. Cell Biol. **141:** 483–492

41 Frazier J. A. and Field C. M. (1997) Actin cytoskeleton: are FH proteins local organizers? Curr. Biol. **7:** R414–417

42 Swan K. A., Severson A. F., Carter J. C., Martin P. R., Schnabel H., Schnabel R. et al. (1998) cyk-1: a *C. elegans* FH gene required for a late step in embryonic cytokinesis. J. Cell Sci. **111:** 2017–2027

43 Epp J. A. and Chant J. (1997) An IQGAP-related protein controls actin-ring formation and cytokinesis in yeast. Curr. Biol. **7:** 921–929

44 Bashour A. M., Fullerton A. T., Hart M. J. and Bloom G. S. (1997) IQGAP1, a Rac- and Cdc42-binding protein, directly binds and cross-links microfilaments. J. Cell Biol. **137:** 1555–1566

45 Osman M. A. and Cerione R. A. (1998) Iqg1p, a yeast homologue of the mammalian IQGAPs, mediates cdc42p effects on the actin cytoskeleton. J. Cell Biol. **142:** 443–455

46 Fishkind D. J. and Wang Y. L. (1993) Orientation and three-dimensional organization of actin filaments in dividing cultured cells. J. Cell Biol. **123:** 837–848

47 Neujahr R., Heizer C., Albrecht R., Ecke M., Schwartz J. M., Weber I. et al. (1997) Three-dimensional patterns and redistribution of myosin II and actin in mitotic *Dictyostelium* cells. J. Cell Biol. **139:** 1793–1804

48 Moores S. L., Sabry J. H. and Spudich J. A. (1996) Myosin dynamics in live *Dictyostelium* cells. Proc. Natl. Acad. Sci. USA **93:** 443–446

49 Zang J. H., Cavet G., Sabry J. H., Wagner P., Moores S. L. and Spudich J. A. (1997) On the role of myosin-II in cytokinesis: division of *Dictyostelium* cells under adhesive and nonadhesive conditions. Mol. Biol. Cell **8:** 2617–2629

50 Sabry J. H., Moores S. L., Ryan S., Zang J. H. and Spudich J. A. (1997) Myosin heavy chain phosphorylation sites regulate myosin localization during cytokinesis in live cells. Mol. Biol. Cell **8:** 2605–2615

51 Yumura S. and Fukui Y. (1998) Spatiotemporal dynamics of actin concentration during cytokinesis and locomotion in *Dictyostelium*. J. Cell Sci. **111:** 2097–2108

52 Ostrow B. D., Chen P. and Chisholm R. L. (1994) Expression of a myosin regulatory light chain phosphorylation site mutant complements the cytokinesis and developmental defects of *Dictyostelium* RMLC null cells. J. Cell Biol. **127:** 1945–1955

53 Jordan P. and Karess R. (1997) Myosin light chain-activating phosphorylation sites are required for oogenesis in *Drosophila*. J. Cell Biol. **139:** 1805–1819

54 Egelhoff T. T., Lee R. J. and Spudich J. A. (1993) *Dictyostelium* myosin heavy chain phosphorylation sites regulate myosin filament assembly and localization in vivo. Cell **75:** 363–371

55 Egelhoff T. T., Naismith T. V. and Brozovich F. V. (1996) Myosin-based cortical tension in *Dictyostelium* resolved into heavy and light chain-regulated components. J. Muscle Res. Cell Motil. **17:** 269–274

56 Satterwhite L. L., Lohka M. J., Wilson K. L., Scherson T. Y., Cisek L. J., Corden J. L. et al. (1992) Phosphorylation of myosin-II regulatory light chain by cyclin-p34^{cdc2}: a mechanism for the timing of cytokinesis. J. Cell Biol. **118:** 595–605

57 DeBiasio R. L., LaRocca G. M., Post P. L. and Taylor D. L. (1996) Myosin II transport organization and phosphorylation: evidence for cortical flow/solation-contraction coupling during cytokinesis and cell locomotion. Mol. Biol. Cell **7:** 1259–1282

58 Matsumura F., Ono S., Yamakita Y., Totsukawa G. and Yamashiro S. (1998) Specific localization of serine 19 phosphorylated myosin II during cell locomotion and mitosis of cultured cells. J. Cell Biol. **140:** 119–129

59 Nishikawa M., Sellers J. R., Adelstein R. S. and Hidaka H. (1984) Protein kinase C modulates in vitro phosphorylation of the smooth muscle heavy meromyosin by myosin light chain kinase. J. Biol. Chem. **259:** 8808–8814

60 Bengur A. R., Robinson E. A., Appella E. and Sellers J. R. (1987) Sequence of the sites phosphorylated by protein kinase C in the smooth muscle myosin light chain. J. Biol. Chem. **262:** 7613–7617

61 Ikebe M. and Reardon S. (1990) Phosphorylation of bovine platelet myosin by protein kinase C. Biochemistry **29:** 2713–2720

62 Yamakita Y., Yamashiro S. and Matsumura F. (1994) In vivo phosphorylation of regulatory light chain of myosin II during mitosis of cultured cells. J. Cell Biol. **124:** 129–137

63 Mishima M. and Mabuchi I. (1996) Cell cycle-dependent phosphorylation of smooth muscle myosin light chain in sea urchin extracts. J. Biochem. **119:** 906–913

64 Totsukawa G., Himi-Nakamura E., Komatsu S., Iwata K., Tezuka A., Sakai H. et al. (1996) Mitosis-specific phosphorylation of smooth muscle regulatory light chain of myosin II at Ser-1 and/or -2 and Thr-9 in sea urchin egg extract. Cell Struct. Funct. **21:** 475–482

65 De Lozanne A. and Spudich J. A. (1987) Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. Science **236:** 1086–1091

66 Knecht D. A. and Loomis W. F. (1987) Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*. Science **236:** 1081–1086

67 Pollenz R. S., Chen T.-L. L., Trivinos-Lagos L. and Chisholm R. L. (1992) The *Dictyostelium* essential light chain is required for myosin function. Cell **69:** 951–962

68 Chen T. L., Kowalcyzk P. A., Ho G. and Chisholm R. L. (1995) Targeted disruption of the *Dictyostelium* myosin essential light chain gene produces cells defective in cytokinesis and morphogenesis. J. Cell Sci. **108:** 3207–3218

69 Chen P., Ostrow B. D., Tafuri S. R. and Chisholm R. L. (1994) Targeted disruption of the *Dictyostelium* RMLC gene produces cells defective in cytokinesis and development. J. Cell Biol. **127:** 1933–1944

70 Fukui Y., De Lozanne A. and Spudich J. A. (1990) Structure and function of the cytoskeleton of a *Dictyostelium* myosin-defective mutant. J. Cell Biol. **110:** 367–378

71 Neujahr R., Heizer C. and Gerisch G. (1997) Myosin II-independent processes in mitotic cells of *Dictyostelium discoideum*: redistribution of the nuclei, re-arrangement of the actin system and formation of the cleavage furrow. J. Cell Sci. **110:** 123–137

72 Faix J., Steinmetz M., Boves H., Kammerer R. A., Lottspeich F., Mintert U. et al. (1996) Cortexillins, major determinants of cell shape and size, are actin-bundling proteins with a parallel coiled-coil tail. Cell **86:** 631–642

73 Neujahr R., Albrecht R., Kohler J., Matzner M., Schwartz J. M., Westphal M. et al. (1998) Microtubule-mediated centrosome motility and the positioning of cleavage furrows in multinucleate myosin II-null cells. J. Cell Sci. **111:** 1227–1240

74 Burton K. and Taylor D. L. (1997) Traction forces of cytokinesis measured with optically modified elastic substrata. Nature **385:** 450–454
75 Mabuchi I. (1986) Biochemical aspects of cytokinesis. Int. Rev. Cytol. **101:** 175–213
76 Rappaport R. (1986) Establishment of the mechanism of cytokinesis in animal cells. Int. Rev. Cytol. **105:** 245–281
77 Bray D. and White J. G. (1988) Cortical flow in animal cells. Science **239:** 883–888
78 Wheatley S. P. and Wang Y. (1996) Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells. J. Cell Biol. **135:** 981–989
79 Harris A. K. and Gewalt S. L. (1989) Simulation testing of mechanisms for inducing the formation of the contractile ring in cytokinesis. J. Cell Biol. **109:** 2215–2223
80 Sawai T. (1998) Evidences For direct involvement of microtubules in cleavage furrow formation in newt eggs. Zoolog. Sci. **15:** 51–56
81 Raich W. B., Moran A. N., Rothman J. H. and Hardin J. (1998) Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein ZEN-4. Mol. Biol. Cell **9:** 2037–2049
82 Fishkind D. J., Silverman J. D. and Wang Y. L. (1996) Function of spindle microtubules in directing cortical movement and actin filament organization in dividing cultured cells. J. Cell Sci. **109:** 2041–2051
83 Canman J. C. and Bement W. M. (1997) Microtubules suppress actomyosin-based cortical flow in *Xenopus* oocytes. J. Cell Sci. **110:** 1907–1917
84 Bornens M., Paintrand M. and Celati C. (1989) The cortical microfilament system of lymphoblasts displays a periodic oscillatory activity in the absence of microtubules: implications for cell polarity. J. Cell Biol. **109:** 1071–1083
85 von Dassow G. and Schubiger G. (1994) How an actin network might cause fountain streaming and nuclear migration in the syncytial *Drosophila* embryo. J. Cell Biol. **127:** 1637–1653
86 Wheatley S. P., O'Connell C. B. and Wang Y. (1998) Inhibition of chromosomal separation provides insights into cleavage furrow stimulation in cultured epithelial cells. Mol. Biol. Cell **9:** 2173–2184
87 Giansanti M. G., Bonaccorsi S., Williams B., Williams E. V., Santolamazza C., Goldberg M. L. et al. (1998) Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. Genes Dev. **12:** 396–410