

Wound-induced contractile ring: a model for cytokinesis¹

Hassina Darenfed and Craig A. Mandato

Abstract: The actomyosin-based contractile ring is required for several biological processes, such as wound healing and cytokinesis of animal cells. Despite progress in defining the roles of this structure in both wound closure and cell division, we still do not fully understand how an actomyosin ring is spatially and temporally assembled, nor do we understand the molecular mechanism of its contraction. Recent results have demonstrated that microtubule-dependent local assembly of F-actin and myosin-II is present in wound closure and is similar to that in cytokinesis in animal cells. Furthermore, signalling factors such as small Rho GTPases have been shown to be involved in the regulation of actin dynamics during both processes. In this review we address recent findings in an attempt to better understand the dynamics of actomyosin contractile rings during wound healing as compared with the final step of animal cell division.

Key words: actomyosin ring, microtubules, cytokinesis, wound healing.

Résumé : L'anneau contractile à base d'actomyosine est impliqué dans plusieurs processus biologiques dont la cicatrisation et la cytokinèse chez les cellules animales. Malgré les progrès accomplis dans la définition du rôle de ces structures dans la fermeture des lésions et la division cellulaire, nous ne comprenons toujours pas complètement comment l'anneau d'actomyosine est assemblé spatialement et temporellement, pas plus que nous ne comprenons les mécanismes moléculaires de sa contraction. Des résultats récents ont démontré que l'assemblage local de F-actine et de myosine de type II dépendant du microtubule est présent lors de la fermeture des lésions et est similaire à la cytokinèse des cellules animales. Qui plus est, les facteurs de signalisation comme des petites GTPases de la famille Rho se sont révélées impliquées dans la régulation de la dynamique de l'actine durant les deux processus. Dans cette revue, nous abordons de récentes découvertes afin de mieux comprendre la dynamique des anneaux contractiles d'actomyosine durant la cicatrisation comparativement à la dernière étape de la division cellulaire.

Mots clés : anneau d'actomyosine, microtubule, cytokinèse, cicatrisation.

[Traduit par la Rédaction]

The actomyosin contractile ring

The actin cytoskeleton together with associated proteins is characterized by an organizational flexibility that is exemplified in the generation of cell polarity, an essential feature for cell motility (Higgs and Pollard 2001; Pollard and Borisy 2003; Schmidt and Hall 1998), phagocytosis (May and Machesky 2001), cell division (Scholey et al. 2003; Pelham and Chang 2002), and wound healing (Martin and Lewis 1992; Bement et al. 1993). One aspect of actin remodelling is represented by the transient changes in cell morphology. Some of the

most spectacular modulations of cell shape are observed in nerve elongation during development (Hely and Willshaw 1998), the formation of membrane protrusions during cell locomotion (Nobes and Hall 1995; Zicha et al. 2003; Rogers et al. 2004), and the constriction of the actin-rich "purse string" during wound healing (Martin and Lewis 1992; Bement et al. 1993) and cytokinesis (Scholey et al. 2003).

The physical division of a cell into 2 separate nucleated daughter cells is in part owing to the constriction of the contractile ring, the result of force generated by actin and myosin at the equatorial cell cortex. As the ring continues to contract, the increase in the cell's surface area is modulated by the influx of phospholipids to the cell membrane through vesicular transport (Lecuit and Wieschaus 2000). These highly controlled cellular activities represent a remarkable example of the strength and plasticity of the cytoskeleton and reflect, in particular, a prominent role of actin cytoskeleton rearrangements in the regulation of fundamental cellular activities during biological and pathological processes.

It has been illustrated that wound closure in a single cell (Mandato and Bement 2001; 2003) and in embryonic tissue (Brock et al. 1996; Kiehart et al. 2000; Wood et al. 2002) is

Received 16 May 2005. Revision received 12 October 2005. Accepted 21 October 2005. Published on the NRC Research Press Web site at <http://bcf.nrc.ca> on 30 November 2005.

H. Darenfed and C.A. Mandato.² Department of Anatomy and Cell Biology, McGill University, 3640 University Street, Montreal, QC H3A 2B2, Canada.

¹This paper is one of a selection of papers published in this Special Issue, entitled CSBMCB – Cellular dynamics, and has undergone the Journal's usual peer review process.

²Corresponding author (e-mail: craig.mandato@mcgill.ca).

Fig. 1. Actomyosin contractile ring assembly during wound healing and cytokinesis. Schematic diagram indicating known and potential molecular regulators of actomyosin contractile ring assembly at the wound border in *Xenopus* oocyte (A) and during cytokinesis (B). A model for microtubule-dependent organization of signalling factors around the wound borders and at the cell cortex, where they control the initiation of actomyosin ring assembly during wound healing (A) and cytokinesis (B). This regulation is thought to be mediated through the generation of active Rho GTPases. Following wounding or at the onset of cytokinesis, Rho GTPase activity initiates the assembly of the actomyosin contractile arrays through the activation of effector molecules. The wound in A was made in a *Xenopus* oocyte visualized by confocal microscopy showing the actin ring (red) and microtubules (green). Bar = 5 μ m.

driven by the presence of an actomyosin contractile ring. Recent studies have shown that disruption of a single cell induces the assembly of an actomyosin ring, a structure that contracts to close the wound by a contractile ring mechanism (Bement et al. 1999; Mandato and Bement 2001). Actomyosin ring assembly is also important in a variety of biological processes, including tissue movement during dorsal closure in *Drosophila* (Nosseli 1998; Jacinto et al. 2002) and the final step of cell division, in which a contractile actin ring plays a critical role in separating the cytoplasm of the dividing cell (Field et al. 1999; Noguchi et al. 2001). Furthermore, a complex network of forces and tension has been shown to contribute to both wound healing and dorsal closure in living *Drosophila* embryos, a natural process of epithelial closure that resembles in many aspects the wound-induced tissue repair (Kiehart et al. 2000; Hutson et al. 2003). From these findings, it seems that although an actomyosin ring provides the mechanical constituent for this process, various inputs, from inside and outside the cell environment, are needed to regulate tissue repair. A more complex mechanism, by which a coordinated closure of tissue is achieved during the developmental process, may contribute to other induced responses such as wound healing.

It is becoming evident that identifying factors that influence and regulate wound healing might be of critical importance, not only for the development of new treatments for clinical conditions in which wound repair is involved but also for greater comprehension of the molecular and cellular basis of processes such as cytokinesis, cancer metastases, and embryonic tissue formation. Furthermore, an important step to the understanding of complex biological processes is the use of simple model systems in which diverse experimental approaches can be utilised in order to analyse different cellular and molecular mechanisms. The oocyte wound healing model bears striking similarities to current models of cytokinesis (Mandato et al. 2000) and cell locomotion (Rodriguez et al. 2003). It also provides strong support for the notion that many cell motility events can be underlain by conserved cytoplasmic activities. This review outlines recent advances in understanding the mechanisms that govern the formation of the actomyosin contractile ring during wound healing and cytokinesis, focusing in particular on the role of the actomyosin arrays in ring closure.

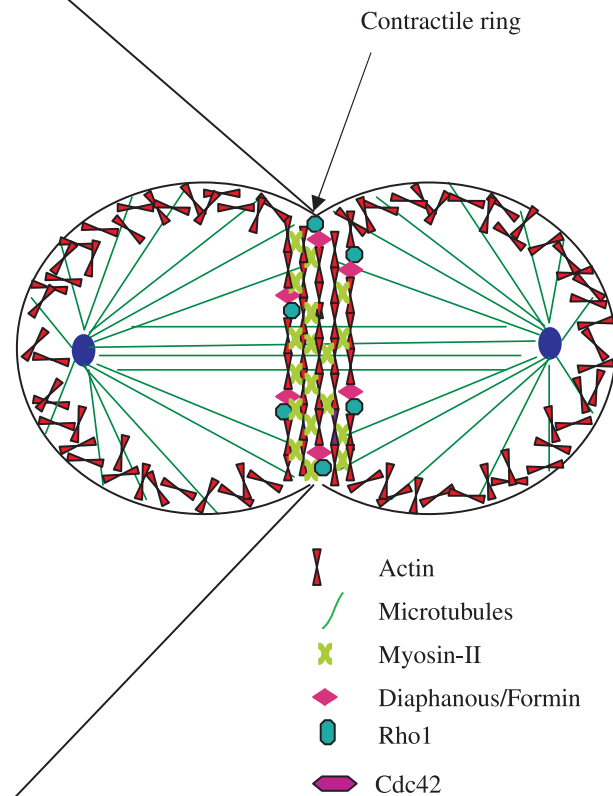
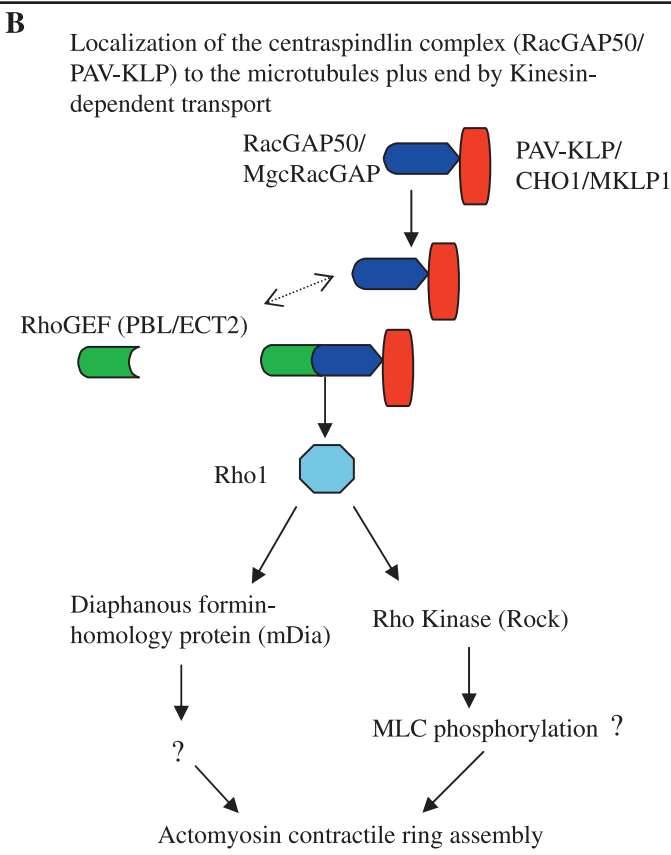
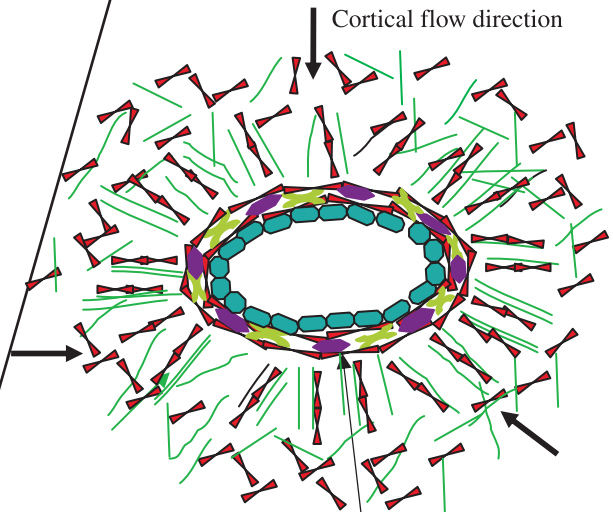
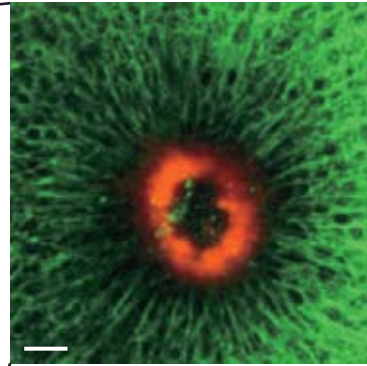
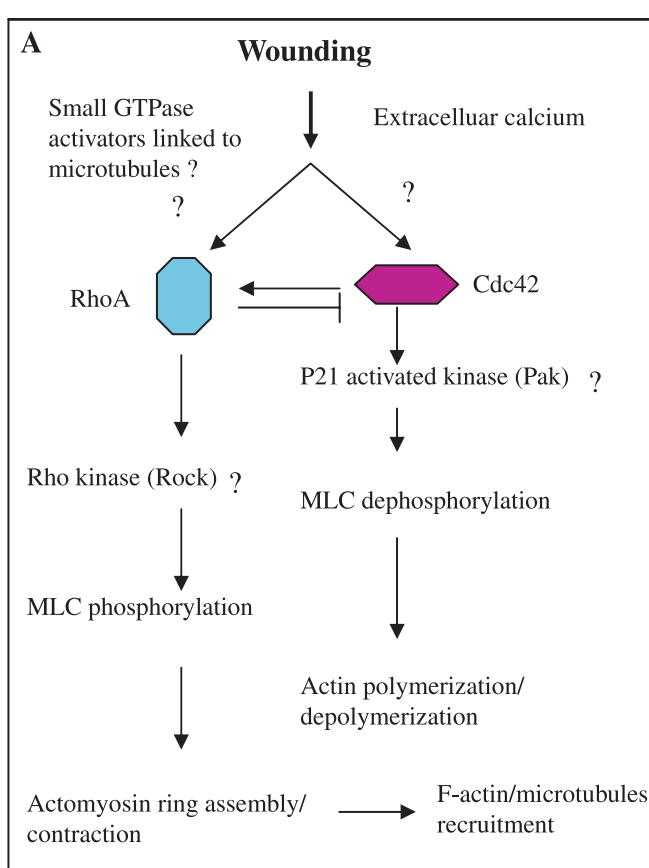
Actomyosin ring: a biological machine adapted to different functions

The generation of an actomyosin contractile ring is required for various biological processes. Its functional involvement has been demonstrated in wound closure (Bement et al. 1993, 1999; Mandato and Bement 2001; Florian et al. 2002), cell division (Field et al. 1999; Noguchi et al. 2001), epithelial movement during morphogenesis (Magie et al. 1999; Harden

et al. 1999; Bloor and Kiehart 2002; Jacinto et al. 2002; Padash Barmchi et al. 2005), endocytosis (Araki et al. 2002; Sokac et al. 2003), and extrusion of apoptotic cells from epithelial cells (Rosenblatt et al. 2001).

Upon wounding of the membrane, *Xenopus* oocytes assemble a ring-like array of actin filaments (F-actin) and myosin-II around the wound, which is encircled by radially organized microtubules (Fig. 1; Mandato and Bement 2003). Four-dimensional imaging with a combination of fluorescent probes and specific manipulations of the cytoskeleton has shown that this array is generated both by assembly of F-actin and myosin-II in specialized zones at the wound border as well as by myosin-driven contraction of stable F-actin at the wound edge (Mandato and Bement 2001). Microtubules are located at the wound border as a result of their association with moving F-actin and are also assembled at the wound border independently of the actin cytoskeleton (Mandato and Bement 2003). Just as the recruitment of microtubules to the wound edge is in part due to actomyosin-based motility, the microtubules, in turn, control the local assembly of actin and myosin-II. The process of cortical flow is thought to create a positive feedback loop, as incoming actin increases contractility, thus increasing cortical flow. Many of the processes observed during oocyte wound healing are consistent with the proposed models of cytokinesis, in which myosin-II accumulates in the cleavage furrow, where it assembles with F-actin into a contractile ring and provides the force for constricting the equator of the cell.

Furthermore, it has been shown that the ring contraction (during *Xenopus* wound healing) can be the result of forces generated by the dynamic assembly of F-actin (Mandato and Bement 2001; Henson et al. 2002; Pelham and Chang 2002; Sokac et al. 2003). Studies of dorsal closure in *Drosophila* and other similar tissue movements in different organisms have revealed that morphogenetic rearrangements of tissues during development are based on similar changes in cytoskeleton organization. In particular, the assembly and contraction of actomyosin rings have been shown to play a critical role in various morphogenetic events (Simske and Hardin 2001; Jacinto et al. 2000, 2002; Harden et al. 2002; Lee and Goldstein 2003). Experimental evidence has shown that contraction of the ingressing cells during *Caenorhabditis elegans* gastrulation is necessary for positioning of blastomeres in developing embryos. This ingression is believed to be powered by the actomyosin-based contraction of the apical side of ingressing cells (Lee and Goldstein 2003). Additionally, morphogenetic events regulating changes in cell shape of *C. Elegans* embryo, such as elongation, are also modulated by the action of actomyosin structures (Simske and Hardin 2001). In *Drosophila* embryos, the contraction of an actomyosin ring and the amnioserosa contributes to the fusion of epithelial layers during dorsal closure (Jacinto et al. 2000).



The process of animal cell cytokinesis is largely mediated by the contractile ring, which begins to form in late anaphase through a reorganization of actin and myosin in the cell cortex. According to this model (Rappaport 1969), astral microtubules emanating from centrosomes provide a cleavage stimulus. It is hypothesized that proteins transported along microtubules from both poles would accumulate in the equator and result in assembly of the contractile ring and subsequent furrowing. Hence, the implication of the contractile actomyosin ring is required for completion of cytokinesis in animal cells (Rappaport 1969; Robinson and Spudich 2000; Glotzer 2001). This dynamic structure assembles at the cell cortex and constricts to ensure that there is equal partitioning of the cytoplasmic organelles. The major result of this highly coordinated process is the separation of the 2 daughter cells at the end of the cell cycle. This is made possible by the ingression of the cleavage furrow composed of actin, myosin-II, and other proteins that link the contractile ring to the cell membrane and regulate its action. While it has been demonstrated that the essential force for the actomyosin ring contraction is generated by myosin-II motor activity (Karess et al. 1991; Bezanilla et al. 1997; Naqvi et al. 2000), recent studies suggest that accumulation and maintenance of myosin-II heavy chain to the cleavage furrow is independent of actin filaments (Naqvi et al. 1999; Motegi et al. 2000).

Despite the identification of a number of proteins regulating the assembly of the cytokinetic ring (Shannon and Li 1999; Somma et al. 2002; Somers and Saint 2003; D'Avino et al. 2004), the molecular mechanisms by which microtubules could influence furrow formation and the contractile ring assembly remain unclear. Among unanswered questions are how this machinery is assembled and disassembled, and how specific proteins cooperate to regulate its action in a precise way during cytokinesis. It is not unrealistic to consider that the constriction of the cytokinetic ring, the closure of induced wounds, and the zipping of epithelial layers during morphogenic development share the mechanical basis associated with cytoskeleton morphological changes (Bement et al. 1999; Wood et al. 2002; Mandato and Bement 2003; Martin and Parkhurst 2004). Therefore, it is plausible to envision similarities between the molecular regulators modulating the assembly and function of the actomyosin ring structure during animal cell cytokinesis and those involved in the repair of inducible cell wound.

In spite of the recent progress in the field of cytokinesis, we still do not understand precisely how the contractile ring is spatially and temporally initiated in a dividing cell, and what the molecular pathways are that regulate its mechanistic constriction. The answers to these questions may help to advance our understanding of the complex process of creating 2 new cells. The use of model systems would allow the molecular dissection and analysis of this highly rapid phenomenon through a direct cell manipulation, since biochemical approaches have proved to be less satisfying for the molecular characterization of cytokinesis. In addition, it will be interesting to define how potential interactions between the actomyosin ring and other cytoskeletal components modulate its assembly and function.

Recent studies have raised questions about the role of microtubules during actomyosin ring assembly. In fact, the results indicate dynamic interactions between microtubules

and actin filaments in the *Xenopus* oocytes model and in cultured cells, and suggest a microtubule-dependent regulation of actomyosin cortical flow (Mandato and Bement 2003; Wheatley et al. 1998; Canman and Bement 1997; Canman et al. 2003). On the other hand, previous data have suggested that while microtubules are only required for furrow initiation in *Dictyostelium* (Neujahr et al. 1998), they play a critical role during contraction and completion of the cleavage furrow in *C. elegans* embryo (Raich et al. 1998). In earlier work, it has been shown that cytokinesis involves not only contraction and cortical assembly but also relaxation or disassembly of the equatorial cortex (Schroeder 1972). The disassembly of actin filaments appears to be an essential step, since cleavage is inhibited by the mutation of cofilin (Gunsalus et al. 1995), a protein mediating actin depolymerization and fragmentation. Moreover, a subset of actin filaments is found to associate end-on with the membrane. This subset is particularly relevant, since forces exerted on these filaments would lead directly to the ingression of the membrane. Upon cytochalasin treatment, cortical actin filaments recoil in random directions throughout the cortex, suggesting that the entire cortex is loaded with forces (Wang et al. 1994). Data supporting this notion suggest that global balance of contractile forces, cortical stiffness, and structural integrity constitute an important factor during cytokinesis. Presumably, myosin-II is involved in both the generation of forces along the equator and in the cortical disassembly process essential for ingression (Guha et al. 2005).

It has been established that the "purse-string" mechanism is the conserved method by which cells, ranging from yeast and slime molds to animal cells, divide (Fishkind and Wang 1995; Glotzer 1997). Nonetheless, studies of cytokinesis in the mold *Dictyostelium discoideum* have revealed that, in addition to using the purse string mechanism model, cells are able to divide using a novel mechanism referred to as adhesion-dependent cytokinesis (Kanada et al. 2005). This mechanism does not depend on myosin-II and is induced when *Dictyostelium* cells are cultured on substrates (Neujahr et al. 1997). It is still uncertain whether such a mechanism is used by animal cells and under what conditions. However, experimental data suggest that additional mechanisms might be used to induce equatorial furrowing in animal cells (O'Connell et al. 1999; Zurek et al. 1990; Somma et al. 2002; Kanada et al. 2005). This is supported by the observations that injection of C3 transferase into adherent mitotic cells induced ectopic furrows without accumulation of myosin-II, whereas the furrow was not induced in non-adherent cells (O'Connell et al. 1999). Moreover, experimental data indicate that adherent normal rat kidney (NRK) and HT1080 cells treated with blebbistatin, a myosin II inhibitor, formed equatorial furrowing (Kanada et al. 2005). Microinjection of antibodies directed against myosin-II into epitheloid kidney cells delayed cytokinesis (Zurek et al. 1990), whereas RNAi inhibition of myosin-II regulatory light-chain expression only partially inhibited cytokinesis in *Drosophila* S2 cells (Somma et al. 2002). In addition, the application of cytochalasin D, which inhibits F-actin polymerization, to the equatorial region of NRK cells accelerated cleavage furrow; however, application to the polar regions completely inhibited cleavage formation (O'Connell et al. 2001).

These observations suggest that cytokinesis in animal cells

can be completed in the absence of myosin-II - dependent contractile activity in the equatorial region. It also points to the role of cell adhesion in the absence of myosin-II as an alternative mechanism used by dividing cells. By contrast, the role of myosin-II - dependent constriction of the contractile ring has been shown in cultured *Xenopus* tissue cells and HeLa cells treated with blebbistatin (Straight et al. 2003). Nevertheless, the possibility that other aspects of myosin-II function during cytokinesis might be altered, causing inhibition of furrow constriction in these cells, cannot be excluded. Thus, these findings point to the complexity of the interdependent network of regulatory pathways leading to cytokinesis.

On the other hand, it is important to consider and evaluate different model organisms in order to better understand the nature of the crosstalk between cytoskeleton components and the actomyosin ring that regulates the process of cytokinesis. From our results we have identified similarities in the dynamic interactions between microtubules and actin cables during single-cell wound closure and cytokinesis (Mandato and Bement 2003). To overcome the difficulties associated with cultured cell cytokinesis experiments, we have used the *Xenopus* oocyte wound system, which offers a powerful model to further characterize the interactions between microtubules and F-actin in order to understand the mechanism of actomyosin ring assembly.

Actomyosin ring in single-cell wound closure and cytokinesis: a dynamic process with multiple players

Actomyosin contractile arrays: assembly and dynamics during wound healing and cytokinesis

It has been well documented that the disruption of cell membrane induces a repair response in animal cells. This natural process is characterized by rapid calcium-dependent membrane fusion events resulting in the generation of an internal membrane linked to the plasma membrane, which seals the site of injury (Terasaki et al. 1997; McNeil et al. 2000). A number of recent results suggest that in addition to the resealing of damaged plasma membrane following disruption, a more complex network of different cytoskeletal proteins and organelles is involved in the restoration of a functional cortex in different cell types.

For example, laser-induced wounds in *Xenopus* oocytes trigger actin polymerization and actin contraction around the damage site (Mandato and Bement 2001, 2003). The de novo synthesized actin and myosin II zones bordering the wound site are assembled into an actomyosin ring that contracts by a contractile mechanism to close the wound. It has been demonstrated that the actomyosin ring assembled around the wound border is calcium dependent and results from the cooperation of 2 distinct pathways that lead to the generation of a flexible but mechanically robust structure (Mandato and Bement 2001). Local actin polymerization around the wound margins and cortical flow of stable F-actin to the ring are responsible for the assembly and maintenance of actin accumulation and the stability of the contractile unit ring. In contrast, accumulation of myosin-II at the site of contraction has been shown to be independent of cortical flow and contractility, although these 2 activities are essential for myosin II to form a functional continuous ring that will close the

wound. Interestingly, a positive feedback loop has been suggested following the observation that the depletion of cortical F-actin surrounding the wound border promotes rapid recruitment of F-actin and consequently results in more actin depletion (Mandato et al. 2000; Mandato and Bement 2001). This hypothesis highlights the potential contribution of localized actin network disassembly in the organization of a contractile actomyosin ring during oocyte wound closure, as has been suggested for other processes such as cytokinesis and cell movement (Gunsalus et al. 1995; DeBiasio et al. 1996; Bretschneider et al. 2002).

In addition to actin and myosin, various other proteins have been shown to modulate contractile ring structure and function with respect to the healing of wounded oocytes. The small GTPases RhoA and Cdc42 are activated around the wound border in *Xenopus* oocytes, forming 2 distinct zones (Fig. 1; Benink and Bement 2005). While RhoA activity is required for F-actin recruitment by cortical flow, its inhibition did not affect local actin assembly. However, the concentration of RhoA on the interior of the contractile ring may regulate myosin-II activity, and Cdc42 activity on the exterior of the ring regulates actin assembly and disassembly (Bement et al. 1999; Benink and Bement 2005). On the other hand, Cdc42 activity is essential for myosin II ring organization, whereas the absence of RhoA inhibits its phosphorylation (Benink and Bement 2005). From these findings, it is becoming more clear that contraction of the actomyosin ring by a purse string mechanism to close the wound is only a part of a more complex and coordinated process involving many different cellular players.

This is also true for the actomyosin ring function during the completion of cytokinesis. Myosin-II, which constitutes the major motor protein for the furrow formation (Straight et al. 2003), is localized to the contractile ring before F-actin in *Xenopus leavis* (Noguchi and Mabuchi 2001), and its activation is regulated by RhoA activity. A variety of other proteins, including the small GTPases and their regulators, have also been identified as regulators of actin dynamics during the assembly of the cytokinetic ring (Larochelle et al. 1996; Drechsel, et al. 1997; Prokopenko et al. 1999; Tatsumoto et al. 2003; Jantsch-Plunger et al. 2000). It seems likely that transient accumulation and activation of Rho GTPase in the cleavage furrow is required for induction, maintenance, and constriction of the contractile ring during cytokinesis (Madaule et al. 1998; Yasui et al. 1998; Di Cunto et al. 2000; Kato et al. 2001).

There are several mechanisms by which Rho GTP could affect the contractile ring. For example, 3 downstream targets of Rho GTP are myosin light chain kinase (MLCK), myosin phosphatase, and ERM family proteins (ezrin, radixin, and moesin) (Kawano et al. 1999). Rho-associated kinase (Rho-kinase) is activated by Rho GTP and can phosphorylate any of these targets. The phosphorylation of MLCK and myosin phosphatase has complementary effects: phosphorylated MLCK activates myosin II, allowing its association with actin filaments, and, conversely, phosphorylation inhibits myosin phosphatase, further upregulating the activation of myosin II (Kawano et al. 1999). ERM family proteins are localized just beneath the plasma membrane and are believed to be involved in the actin filament/plasma membrane association in the cell cortex. They may act as linkers between external

regulators of cytokinesis and the cytoskeleton. It has been demonstrated that activation of the external domain of the cell surface glycoprotein CD44 causes cytoskeletal reorganization through activation of ERM proteins (Tsukita et al. 1997). Further evidence for the roles of these Rho-kinase-regulated proteins during cytokinesis comes from the observation that all 3 components, MLCK, myosin phosphatase, and ERM family proteins, accumulate in the cleavage furrow during cytokinesis (Kawano et al. 1999). Hence, these factors may link Rho activation to contractile ring formation through phosphorylation by Rho-kinase.

The guanine nucleotide exchange factor ECT2, also known as Pebble in *Drosophila*, is localized to the contractile ring, where it activates Rho GTPase, thus promoting furrow induction (Prokopenko et al. 1999). In other model systems the depletion of components of the centralspindlin complex, CYK-4 (GAP) or ZEN-4, a kinesin-like protein, inhibits the completion of cytokinesis in *C. elegans* and *Drosophila* S2 cells (Lehner et al. 1992; Powers et al. 1998; Raich et al. 1998; Jantsch-Plunger et al. 2000; Somma et al. 2002). Recent studies have provided evidence indicating that effectors of the Rho GTPase, such as Diaphanous formin-homology protein (Afshar et al. 2000; Kato et al. 2001) and some members of Rho-dependent kinases (Madaule et al. 1998; Kosako et al. 2000), are involved in the molecular pathway through which RhoA modulates actin reorganization during cytokinesis. In addition, proteins responsible for actin depolymerization, such as cofilin, have also been shown to play a role in the modulation of cytokinetic ring organization (Gunsalus et al. 1995; Ono et al. 2003; Hotulainen et al. 2005).

Membrane interactions with actin cytoskeleton also play a crucial role in actin reorganization during cytokinesis. Studies have shown that phosphatidylinositol (PtdIns) play a role in the regulation of contractile ring stability (Saul et al. 2004; Wong et al. 2005). Indeed, PtdIns(4,5)P₂ hydrolysis is required for the recruitment of polymerized actin from the cell poles to the furrow in crane fly spermatocytes and *Drosophila* spermatocytes (Saul et al. 2004; Wong et al. 2005). A recent study demonstrates the accumulation of PIP5Kb and PI(4,5)P₂ at the cleavage furrow of dividing cells, where the local generation of PI(4,5)P₂ is required for the proper completion of cytokinesis (Emoto et al. 2005). It was suggested that RhoA activates PIPK5b at the cleavage furrow where the colocalization of the 2 proteins was observed. This led to local production of PI(4,5)P₂ at the furrow membrane (Emoto et al. 2005). Moreover, a membrane domain with a particular lipid composition is formed at the cleavage furrow membrane and may regulate the actin contractile ring rearrangement during late cytokinesis through the binding of actin regulatory proteins (Emoto et al. 2005). From these observations, it is plausible to envision that some molecular regulators of actomyosin ring assembly might be conserved during wound healing and cytokinesis.

Microtubule reorganization modulates the assembly and dynamics of the actomyosin ring in cytokinesis and single-cell wound

Coordination between microtubule and F-actin networks has been shown to be involved in different cellular activities. The reorganization of F-actin and myosin II at the cell equator

in several biological systems is a key event in the process of furrow initiation and ingression. Several sources of data indicate that microtubule structures are implicated in actomyosin ring formation and furrow ingression, but the precise molecular events that regulate the assembly and contraction of the ring during cytokinesis are not well understood. Recent studies suggest a signal-based regulation of these interactions, which are critical for the completion of cytokinesis. This regulation is thought to be mediated by microtubules interacting with the cell cortex to ensure that the molecular signals essential for this aspect of cytokinesis are delivered (Murata-Hori and Wang 2002; Bringmann and Hyman 2005; Robinson and Spudich 2000; D'Avino et al. 2005).

The role of different microtubule structures in the modulation of the cleavage furrow has been examined in several experimental systems (Powers et al. 1998; Kurz et al. 2002; Shuster and Burgess 2002; Canman et al. 2003; Minestrini et al. 2003; Alsop and Zhang 2003; Inoue et al. 2004; Martin et al. 2005). These studies have led to various hypotheses of how microtubules might modulate actomyosin ring contraction and furrow ingression. These differences might reflect the variations among the experimental models and approaches, as well as the diversity in the mechanisms by which microtubule dynamics may modulate furrow ingression in different cell types (Wang 2001). In addition, several experiments have demonstrated that there is a high level of stable microtubules in the position of furrow formation in tissue culture cells. This suggests that the stable microtubules associated with the chromosomes are responsible for the induction of furrowing at the cell cortex, whereas a more dynamic microtubule population might inhibit furrow induction in other cell regions (Canman et al. 2003). These studies support the notion that changes in the dynamics of microtubule structures in different locations within the cell regulate the actomyosin assembly and furrow progression. As a consequence, another theory has been proposed to integrate various data obtained from different organisms. This new view emphasizes the importance of the dynamics of different microtubule structures during furrow ingression (Glotzer 2004). It has been proposed that more stable microtubules promote actomyosin assembly, whereas dynamic populations of these structures inhibit furrow ingression.

At a molecular level, a highly conserved signalling complex, centralspindlin, is localized to the astral microtubules and the central spindle, and has been shown to be involved in furrow formation and ingression. Importantly, recent studies in *Drosophila* embryos and mammals indicate that Pavarotti kinesin-like protein and the GTPase-activating protein RacGAP50C, 2 components of the centralspindlin complex, are implicated in the recruitment of actin to the actomyosin ring and the constriction of the ring, respectively (Minestrini et al. 2003; D'Avino et al. 2004; Yoshizaki et al. 2004). This has led to the hypothesis that microtubule-mediated delivery of this complex to the cell cortex could be 1 mechanism of furrow initiation and ingression (D'Avino et al. 2005).

Hence, although various observations have been obtained from different systems, these experimental findings reinforce the idea of the microtubule-mediated regulation of furrow formation and cleavage. However, these observations raise important unanswered questions. For example, even though

there is increasing evidence supporting the importance of some components of the centralspindlin complex in the assembly of the actomyosin ring, it is still unknown how these molecules achieve their role in a specific space and time, and how their actions are regulated to further allow subsequent disassembly of the ring. Therefore, understanding how the actomyosin ring is assembled and how the ingression of the furrow is initiated and followed by the disassembly of the ring might help to reveal the molecular mechanisms underlying the contribution of the microtubules in the highly regulated step of the cell cycle.

Another aspect of the interplay among microtubules and F-actin associated with actomyosin-based processes can be observed during the closure of single-cell wound. In the *Xenopus* oocyte wound-induced system, the actomyosin ring organization is modulated by microtubule dynamics during wound closure. Our results indicate that assembly of the actomyosin ring in wounded *Xenopus* oocytes is associated with the translocation of microtubules. The translocation of microtubules toward wound edge is actin dependent and also promotes F-actin assembly at the wound edge. It has been shown that the translocated microtubules buckle and break upon reaching the wound edge. Furthermore, expressed eGFP/ α -tubulin showed an active zone of assembly/disassembly around the wound border that coincided with the zone of actin polymerization, implicating the microtubules in actin assembly during wound closure. Four-dimensional time lapse confocal microscopy demonstrated that dynamic microtubules modulate actomyosin ring stability through the coordination of the actin assembly zone with the contractile actomyosin ring (Mandato and Bement 2003). These observations, together with our previous results (Mandato and Bement 2001), have led to a model in which wounding triggers local microtubule assembly around the wound border, which in turn promotes local F-actin assembly.

These coordinated interactions between microtubules and F-actin observed during wound closure in a single cell are also features of other fundamental activities, including growth and differentiation, cell migration, and division. This could reflect a conserved interplay between the 2 cytoskeletal elements, which collaborate to interpret several cellular cues during diverse biological processes. On the basis of our recent findings on the dynamic interplay between these 2 cytoskeletal structures during actomyosin assembly and contraction, we believe that the *Xenopus* oocyte model offers a promising avenue to investigate these interactions at the molecular level and to define the similarities that might exist between the regulation of the wound-induced actomyosin ring and the processes that drive cytokinesis.

Conclusion

Although advancement in the field of cytokinesis has been made over the last few years, we are just starting to understand some of the possible functions of the proteins involved in the regulation of microtubule and actomyosin interactions during furrow induction and ingression. Many approaches used to study the role of the potential regulators of actomyosin ring assembly have been based on genetic modifications, such as mutant generation and the expression of dominant

negative forms of the protein, from which different results are obtained in different organisms. It will be interesting for future studies to apply more sensitive methods, such as time-lapse microscopy combined with gene knockout, to examine the rapid rearrangements of cytoskeleton structures that accompany actomyosin ring formation and to monitor microtubule reorganization during its assembly and contraction.

Understanding the interactions between microtubules and the different molecular regulators that modulate actomyosin ring function during cytokinesis will provide a better understanding of how multiple cellular components cooperate to drive the separation of 2 cells. In our model, there are striking similarities between actin–microtubule interactions during oocyte wound closure and the actomyosin ring contraction that drives cytokinesis, making this model a useful system for studying the interactions between cytoskeleton elements that modulate actomyosin assembly and the molecular factors that regulate its function. Furthermore, some of the molecular regulators of the wound-induced actomyosin have also been involved in the dynamics of the cytokinetic ring. We believe that the strengths of this model are as follows: first, it allows for the direct manipulation of the cell in vivo, therefore real-time observations of the ring assembly and closure can be made. Second, because of the size of the model system the process of actomyosin assembly is less rapid compared with the cytokinetic ring, and this also constitutes an advantage for assessing the spatial and temporal distribution of the molecular regulators of the process. There are evident differences between the single-cell wound closure and the cytokinetic apparatus. Nevertheless, similarities in the early steps of the actomyosin ring assembly suggest that the single-cell wound model might open new avenues to future studies on how microtubules and F-actin interact to coordinate actomyosin ring assembly and dynamics during cytokinesis, and how their interactions may be operating in other actomyosin contractile-based structures.

Acknowledgements

We thank Tong Zhang for supplying the confocal image of *Xenopus* oocyte wound. Thanks to Bama Dayanandan and Mike Logan for critical reading of this manuscript. This research was supported by a Canadian Institutes of Health Research grant to C.A.M. C.A.M. is a Canada Research Chair.

References

- Afshar, K., Stuart, B., and Wasserman, S.A. 2000. Functional analysis of the *Drosophila* diaphanous FH protein in early embryonic development. *Development (Cambridge)*, **127**: 1887–1897.
- Alsop, G.B., and Zhang, D. 2003. Microtubules are the only structural constituent of the spindle apparatus required for induction of cell cleavage. *J. Cell Biol.* **162**: 383–390.
- Araki, M., Takano, T., Uemonsa, T., Nakane, Y., Tsudzuki, M., and Kaneko, T. 2002. Epithelia-mesenchyme interaction plays an essential role in transdifferentiation of retinal pigment epithelium of silver mutant quail: localization of FGF and related molecules and aberrant migration pattern of neural crest cells during eye rudiment formation. *Dev. Biol.* **244**: 358–371.
- Bement, W.M., Forscher P., and Mooseker M.S. 1993. A novel

- cytoskeletal structure involved in purse string wound closure and cell polarity maintenance. *J. Cell Biol.* **121**: 565–578.
- Bement, W.M., Mandato, C.A., and Kirsch, M.N. 1999. Wound-induced assembly and closure of an actomyosin purse string in *Xenopus* oocytes. *Curr. Biol.* **9**: 579–587.
- Benink, H.A., and Bement, W.M. 2005. Concentric zones of active RhoA and Cdc42 around single cell wounds. *J. Cell Biol.* **168**: 429–439.
- Bezanilla, M., Forsburg, S.L., and Pollard, T.D. 1997. Identification of a second myosin-II in *Schizosaccharomyces pombe*: Myp2p is conditionally required for cytokinesis. *Mol. Biol. Cell*, **8**: 2693–2705.
- Bloor, J.W., and Kiehart, D.P. 2002. *Drosophila* RhoA regulates the cytoskeleton and cell-cell adhesion in the developing epidermis. *Development (Cambridge)*, **129**: 3173–3183.
- Bretschneider, T., Jonkman, J., Kohler, J., Medalia, O., Barisic, K., Weber, I. et al. 2002. Dynamic organization of the actin system in the motile cells of *Dictyostelium*. *J. Muscle Res. Cell. Motil.* **23**: 639–649.
- Bringmann, H., and Hyman, A.A. 2005. A cytokinesis furrow is positioned by two consecutive signals. *Nature (London)*, **436**: 731–734.
- Brock, J., Midwinter, K., Lewis, J., and Martin, P. 1996. Healing of incisional wounds in the embryonic chick wing bud: characterization of the actin purse-string and demonstration of a requirement for Rho activation. *J. Cell Biol.* **135**: 1097–1107.
- Canman, J.C., and Bement, W.M. 1997. Microtubules suppress actomyosin-based cortical flow in *Xenopus* oocytes. *J. Cell Sci.* **110**(Pt. 16): 1907–1917.
- Canman, J.C., Cameron, L.A., Maddox, P.S., Straight, A., Tirnauer, J.S., Mitchison, T.J. et al. 2003. Determining the position of the cell division plane. *Nature (London)*, **424**: 1074–1078.
- D'Avino, P.P., Savoian, M.S., and Glover, D.M. 2004. Mutations in *sticky* lead to defective organization of the contractile ring during cytokinesis and are enhanced by Rho and suppressed by Rac. *J. Cell Biol.* **166**: 61–71.
- D'Avino, P.P., Savoian, M.S., and Glover, D.M. 2005. Cleavage furrow formation and ingression during animal cytokinesis: a microtubule legacy. *J. Cell Sci.* **118**: 1549–1558.
- DeBiasio, R.L., LaRocca, G.M., Post, P.L., and Taylor, D.L. 1996. Myosin II transport, organization, and phosphorylation: evidence for cortical flow/solution-contraction coupling during cytokinesis and cell locomotion. *Mol. Biol. Cell.* **7**: 1259–1282.
- Di Cunto, F., Imarisio, S., Hirsch, E., Broccoli, V., Bulfone, A., Migheli, A. et al. 2000. Defective neurogenesis in citron kinase knockout mice by altered cytokinesis and massive apoptosis. *Neuron*, **28**: 115–127.
- 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.
- Emoto, K., Inadome, H., Kanaho, Y., Narumiya, S., and Umeda, M. 2005. Local change in phospholipid composition at the cleavage furrow is essential for completion of cytokinesis. *J. Biol. Chem.* 14 Sept. [Epub. ahead of print.]
- Field, C., Li, R., and Oegema, K. 1999. Cytokinesis in eukaryotes: a mechanistic comparison. *Curr. Opin. Cell Biol.* **11**: 68–80.
- Fishkind, D.J., and Wang, Y.L. 1995. New horizons for cytokinesis. *Curr. Opin. Cell Biol.* **7**: 23–31.
- Florian, P., Schoneberg, T., Schulzke, J.D., Fromm, M., and Gitter, A.H. 2002. Single-cell epithelial defects close rapidly by an actomyosin purse string mechanism with functional tight junctions. *J. Physiol. (Oxford)*, **545**: 485–499.
- Glotzer, M. 1997. The mechanism and control of cytokinesis. *Curr. Opin. Cell Biol.* **9**: 815–823.
- Glotzer, M. 2001. Animal cell cytokinesis. *Annu. Rev. Cell Dev. Biol.* **17**: 351–386.
- Glotzer, M. 2004. Cleavage furrow positioning. *J. Cell Biol.* **164**: 347–351.
- Guha, M., Zhou, M., and Wang, Y.L. 2005. Cortical actin turnover during cytokinesis requires myosin II. *Curr. Biol.* **15**: 732–736.
- Gunsalus, K.C., Bonaccorsi, S., Williams, E., Verni, F., Gatti, M., and Goldberg, M.L. 1995. Mutations in twinstar, a *Drosophila* gene encoding a cofilin/ADF homologue, result in defects in centrosome migration and cytokinesis. *J. Cell. Biol.* **131**: 1243–1259.
- Harden, N., Ricos, M., Ong, Y.M., Chia, W., and Lim, L. 1999. Participation of small GTPases in dorsal closure of the *Drosophila* embryo: distinct roles for Rho subfamily proteins in epithelial morphogenesis. *J. Cell Sci.* **112**(Pt. 3): 273–284.
- Harden, N., Ricos, M., Yee, K., Sanny, J., Langmann, C., Yu, H. et al. 2002. Drac1 and Crumbs participate in amnioserosa morphogenesis during dorsal closure in *Drosophila*. *J. Cell Sci.* **115**: 2119–2129.
- Hely, T.A., and Willshaw, D.J. 1998. Short-term interactions between microtubules and actin filaments underlie long-term behaviour in neuronal growth cones. *Proc. Biol. Sci. R. Soc. Lond.* **265**: 1801–1807.
- Henson, J.H., Nazarian, R., Schulberg, K.L., Trabosh, V.A., Kolnik, S.E., Burns, A.R., and McPartland K.J. 2002. Wound closure in the lamellipodia of single cells: mediation by actin polymerization in the absence of an actomyosin purse string. *Mol. Biol. Cell.* **13**: 1001–1014.
- Higgs, H.N., and Pollard, T.D. 2001. Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. *Annu. Rev. Biochem.* **70**: 649–676.
- Hotulainen, P., Paunola, E., Vartiainen, M.K., and Lappalainen, P. 2005. Actin-depolymerizing factor and cofilin-1 play overlapping roles in promoting rapid F-actin depolymerization in mammalian nonmuscle cells. *Mol. Biol. Cell.* **16**: 649–664.
- Hutson, M.S., Tokutake, Y., Chang, M.S., Bloor, J.W., Venakides, S., Kiehart, D.P., and Edwards, G.S. 2003. Forces for morphogenesis investigated with laser microsurgery and quantitative modeling. *Science (Washington, D.C.)*. **300**: 145–149.
- Inoue, Y.H., Savoian, M.S., Suzuki, T., Mathe, E., Yamamoto, M.T., and Glover, D.M. 2004. Mutations in orbit/mast reveal that the central spindle is comprised of two microtubule populations, those that initiate cleavage and those that propagate furrow ingression. *J. Cell Biol.* **166**: 49–60.
- Jacinto, A., Wood W., Balayo T., Turmaine M., Martinez-Arias A., and Martin P. 2000. Dynamic actin-based epithelial adhesion and cell matching during *Drosophila* dorsal closure. *Curr Biol.* **10**: 1420–1426.
- Jacinto, A., Wood, W., Woolner, S., Hiley, C., Turner, L., Wilson, C. et al. 2002. Dynamic analysis of actin cable function during *Drosophila* dorsal closure. *Curr. Biol.* **12**: 1245–1250.
- Jantsch-Plunger, V., Gonczy, P., Romano, A., Schnabel, H., Hamill, D., Schnabel, R. et al. 2000. CYK-4: A Rho family gtpase activating protein (GAP) required for central spindle formation and cytokinesis. *J. Cell Biol.* **149**: 1391–1404.
- Kanada, M., Nagasaki, A., and Uyeda, T.Q. 2005. Adhesion-dependent and contractile ring-independent equatorial furrowing during cytokinesis in mammalian cells. *Mol. Biol. Cell*, **16**: 3865–3872.
- Karess, R.E., Chang, X.J., Edwards, K.A., Kulkarni, S., Aguilera, I., and Kiehart, D.P. 1991. The regulatory light chain of nonmuscle myosin is encoded by spaghetti-squash, a gene required for cytokinesis in *Drosophila*. *Cell*, **65**: 1177–1189.
- Kato, T., Watanabe, N., Morishima, Y., Fujita, A., Ishizaki, T., and

- Narumiya, S. 2001. Localization of a mammalian homolog of diaphanous, mDia1, to the mitotic spindle in HeLa cells. *J. Cell Sci.* **114**: 775–784.
- Kawano, Y., Fukata, Y., Oshiro, N., Amano, M., Nakamura, T., Ito, M. et al. 1999. Phosphorylation of myosin-binding subunit (MBS) of myosin phosphatase by Rho-kinase in vivo. *J. Cell Biol.* **147**: 1023–1038.
- Kiehart, D.P., Galbraith, C.G., Edwards, K.A., Rickoll, W.L., and Montague, R.A. 2000. Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. *J. Cell Biol.* **149**: 471–490.
- Kosako, H., Yoshida, T., Matsumura, F., Ishizaki, T., Narumiya, S., and Inagaki, M. 2000. Rho-kinase/ROCK is involved in cytokinesis through the phosphorylation of myosin light chain and not ezrin/radixin/moesin proteins at the cleavage furrow. *Oncogene*, **19**: 6059–6064.
- Kurz, T., Pintard, L., Willis, J.H., Hamill, D.R., Gonczy, P., Peter, M., and Bowerman, B. 2002. Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. *Science* (Washington, D.C.), **295**: 1294–1298.
- Larochelle, D.A., Vithalani, K.K., and De Lozanne, A. 1996. A novel member of the rho family of small GTP-binding proteins is specifically required for cytokinesis. *J. Cell Biol.* **133**: 1321–1329.
- Lecuit, T., and Wieschaus, E. 2000. Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the *Drosophila* embryo. *J. Cell Biol.* **150**: 849–860.
- Lee, J.Y., and Goldstein, B. 2003. Mechanisms of cell positioning during *C. elegans* gastrulation. *Development* (Cambridge), **130**: 307–320.
- Lehner, C.F., Ried, G., Stern, B., and Knoblich, J.A. 1992. Cyclins and cdc2 kinases in *Drosophila*: genetic analyses in a higher eukaryote. *Ciba Found. Symp.* **170**: 97–109; discussion 110–114.
- 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* (London), **394**: 491–494.
- Magie, C.R., Meyer, M.R., Gorsuch, M.S., and Parkhurst, S.M. 1999. Mutations in the Rho1 small GTPase disrupt morphogenesis and segmentation during early *Drosophila* development. *Development* (Cambridge), **126**: 5353–5364.
- Mandato, C.A., and Bement, W.M. 2001. Contraction and polymerization cooperate to assemble and close actomyosin rings around *Xenopus* oocyte wounds. *J. Cell Biol.* **154**: 785–797.
- Mandato, C.A., and Bement, W.M. 2003. Actomyosin transports microtubules and microtubules control actomyosin recruitment during *Xenopus* oocyte wound healing. *Curr. Biol.* **13**: 1096–1105.
- Mandato, C.A., Benink, H.A., and Bement, W.M. 2000. Microtubule-actomyosin interactions in cortical flow and cytokinesis. *Cell Motil. Cytoskelet.* **45**: 87–92.
- Martin, P., and Lewis, J. 1992. Actin cables and epidermal movement in embryonic wound healing. *Nature* (London), **360**: 179–183.
- Martin, P., and Parkhurst, S.M. 2004. Parallels between tissue repair and embryo morphogenesis. *Development* (Cambridge), **131**: 3021–3034.
- Martin, S.G., McDonald, W.H., Yates, J.R., 3rd, and Chang, F. 2005. Tea4p links microtubule plus ends with the formin for3p in the establishment of cell polarity. *Dev. Cell.* **8**: 479–491.
- May, R.C., and Machesky, L.M. 2001. Phagocytosis and the actin cytoskeleton. *J. Cell Sci.* **114**: 1061–1077.
- McNeil, P.L., Vogel, S.S., Miyake, K., and Terasaki, M. 2000. Patching plasma membrane disruptions with cytoplasmic membrane. *J. Cell. Sci.* **113**(Pt. 11): 1891–1902.
- Minestrini, G., Harley, A.S., and Glover, D.M. 2003. Localization of Pavarotti-KLP in living *Drosophila* embryos suggests roles in reorganizing the cortical cytoskeleton during the mitotic cycle. *Mol. Biol. Cell.* **14**: 4028–4038.
- Motegi, F., Nakano, K., and Mabuchi, I. 2000. Molecular mechanism of myosin-II assembly at the division site in *Schizosaccharomyces pombe*. *J. Cell Sci.* **113**(Pt. 10): 1813–1825.
- Murata-Hori, M., and Wang, Y.L. 2002. Both midzone and astral microtubules are involved in the delivery of cytokinesis signals: insights from the mobility of aurora B. *J. Cell Biol.* **159**: 45–53.
- Naqvi, N.I., Eng, K., Gould, K.L., and Balasubramanian, M.K. 1999. Evidence for F-actin-dependent and -independent mechanisms involved in assembly and stability of the medial actomyosin ring in fission yeast. *EMBO J.* **18**: 854–862.
- Naqvi, N.I., Wong, K.C., Tang, X., and Balasubramanian, M.K. 2000. Type II myosin regulatory light chain relieves auto-inhibition of myosin-heavy-chain function. *Nat. Cell Biol.* **2**: 855–858.
- 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**(Pt. 2): 123–137.
- Neujahr, R., Albrecht, R., Kohler, J., Matzner, M., Schwartz, J.M., Westphal, M., and Gerisch, G. 1998. Microtubule-mediated centrosome motility and the positioning of cleavage furrows in multinucleate myosin II-null cells. *J. Cell Sci.* **111**(Pt. 9): 1227–1240.
- Nobes, C.D., and Hall, A. 1995. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell.* **81**: 53–62.
- Noguchi, T., Arai, R., Motegi, F., Nakano, K., and Mabuchi, I. 2001. Contractile ring formation in *Xenopus* egg and fission yeast. *Cell Struct. Funct.* **26**: 545–554.
- Noguchi, T., and Mabuchi, I. 2001. Reorganization of actin cytoskeleton at the growing end of the cleavage furrow of *Xenopus* egg during cytokinesis. *J. Cell Sci.* **114**: 401–412.
- O'Connell, C.B., Wheatley, S.P., Ahmed, S., and Wang, Y.L. 1999. The small GTP-binding protein rho regulates cortical activities in cultured cells during division. *J. Cell Biol.* **144**: 305–313.
- O'Connell, C.B., Warner, A.K., and Wang, Y. 2001. Distinct roles of the equatorial and polar cortices in the cleavage of adherent cells. *Curr. Biol.* **11**: 702–707.
- Ono, K., Parast, M., Alberico, C., Benian, G.M., and Ono, S. 2003. Specific requirement for two ADF/cofilin isoforms in distinct actin-dependent processes in *Caenorhabditis elegans*. *J. Cell Sci.* **116**: 2073–2085.
- Padash Barmchi, M., Rogers, S., and Hacker, U. 2005. DRhoGEF2 regulates actin organization and contractility in the *Drosophila* blastoderm embryo. *J. Cell Biol.* **168**: 575–585.
- Pelham, R.J., and Chang, F. 2002. Actin dynamics in the contractile ring during cytokinesis in fission yeast. *Nature* (London), **419**: 82–86.
- Pollard, T.D., and Borisy, G.G. 2003. Cellular motility driven by assembly and disassembly of actin filaments. *Cell.* **112**: 453–465.
- Powers, J., Bossinger, O., Rose, D., Strome, S., and Saxton, W. 1998. A nematode kinesin required for cleavage furrow advancement. *Curr. Biol.* **8**: 1133–1136.
- Prokopenko, S.N., Brumby, A., O'Keefe, L., Prior, L., He, Y., Saint, R., and Bellen, H.J. 1999. A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes Dev.* **13**: 2301–2314.
- 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.

- Rappaport, R. 1969. Division of isolated furrows and furrow fragments in invertebrate eggs. *Exp. Cell Res.* **56**: 87–91.
- Robinson, D.N., and Spudich, J.A. 2000. Towards a molecular understanding of cytokinesis. *Trends Cell Biol.* **10**: 228–237.
- Rodriguez, O.C., Schaefer, A.W., Mandato, C.A., Forscher, P., Bement, W.M., and Waterman-Storer, C.M. 2003. Conserved microtubule-actin interactions in cell movement and morphogenesis. *Nat. Cell Biol.* **5**: 599–609.
- Rogers, S.L., Wiedemann, U., Hacker, U., Turck, C., and Vale, R.D. 2004. *Drosophila* RhoGEF2 associates with microtubule plus ends in an EB1-dependent manner. *Curr. Biol.* **14**: 1827–1833.
- Rosenblatt, J., Raff, M.C., and Cramer, L.P. 2001. An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism. *Curr. Biol.* **11**: 1847–1857.
- Saul, D., Fabian L., Forer, A., and Brill, J.A. 2004. Continuous phosphatidylinositol metabolism is required for cleavage of crane fly spermatocytes. *J. Cell Sci.* **117**: 3887–3896.
- Schmidt, A., and Hall, M.N. 1998. Signaling to the actin cytoskeleton. *Annu. Rev. Cell Dev. Biol.* **14**: 305–338.
- Scholey, J.M., Brust-Mascher, I., and Mogilner, A. 2003. Cell division. *Nature (London)*, **422**: 746–752.
- Schroeder, T.E. 1972. The contractile ring. II. Determining its brief existence, volumetric changes, and vital role in cleaving *Arbacia* eggs. *J. Cell Biol.* **53**: 419–434.
- Shannon, K.B., and Li, R. 1999. The multiple roles of Cyk1p in the assembly and function of the actomyosin ring in budding yeast. *Mol. Biol. Cell.* **10**: 283–296.
- Shuster, C.B., and Burgess, D.R. 2002. Targeted new membrane addition in the cleavage furrow is a late, separate event in cytokinesis. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 3633–3638.
- Simske, J.S., and Hardin, J. 2001. Getting into shape: epidermal morphogenesis in *Caenorhabditis elegans* embryos. *Bioessays.* **23**: 12–23.
- Sokac, A.M., Co, C., Taunton, J., and Bement, W. 2003. Cdc42-dependent actin polymerization during compensatory endocytosis in *Xenopus* eggs. *Nat. Cell Biol.* **5**: 727–732.
- Somers, W.G., and Saint, R. 2003. A RhoGEF and Rho family GTPase-activating protein complex links the contractile ring to cortical microtubules at the onset of cytokinesis. *Dev. Cell.* **4**: 29–39.
- Somma, M.P., Fasulo, B., Cenci, G., Cundari, E., and Gatti, M. 2002. Molecular dissection of cytokinesis by RNA interference in *Drosophila* cultured cells. *Mol. Biol. Cell.* **13**: 2448–2460.
- Straight, A.F., Cheung, A., Limouze, J., Chen, I., Westwood, N.J., Sellers, J.R., and Mitchison, T.J. 2003. Dissecting temporal and spatial control of cytokinesis with a myosin II Inhibitor. *Science (Washington, D.C.)*, **299**: 1743–1747.
- Tatsumoto, T., Sakata, H., Dasso, M., and Miki, T. 2003. Potential roles of the nucleotide exchange factor ECT2 and Cdc42 GTPase in spindle assembly in *Xenopus* egg cell-free extracts. *J. Cell. Biochem.* **90**: 892–900.
- Terasaki, A.G., Ohnuma, M., and Mabuchi, I. 1997. Identification of actin-binding proteins from sea urchin eggs by F-actin affinity column chromatography. *J. Biochem. (Tokyo)*, **122**: 226–236.
- Tsukita, S., Yonemura, S., and Tsukita, S. 1997. ERM proteins: head-to-tail regulation of actin-plasma membrane interaction. *Trends Biochem. Sci.* **22**: 53–58.
- Wang, Y.L. 2001. The mechanism of cytokinesis: reconsideration and reconciliation. *Cell Struct. Funct.* **26**: 633–638.
- Wang, Y.L., Silverman, J.D., and Cao, L.G. 1994. Single particle tracking of surface receptor movement during cell division. *J. Cell Biol.* **127**: 963–971.
- 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.
- Wong, R., Hadjiyanni, I., Wei, H.C., Polevoy, G., McBride, R., Sem, K.P., and Brill, J.A. 2005. PIP2 hydrolysis and calcium release are required for cytokinesis in *Drosophila* spermatocytes. *Curr. Biol.* **15**: 1401–1406.
- Wood, W., Jacinto, A., Grose, R., Woolner, S., Gale, J., Wilson C., and Martin P. 2002. Wound healing recapitulates morphogenesis in *Drosophila* embryos. *Nat. Cell Biol.* **4**: 907–912.
- Yasui, Y., Amano, M., Nagata, K., Inagaki, N., Nakamura, H., Saya, H. et al. 1998. Roles of Rho-associated kinase in cytokinesis; mutations in Rho-associated kinase phosphorylation sites impair cytokinetic segregation of glial filaments. *J. Cell Biol.* **143**: 1249–1258.
- Yoshizaki, H., Ohba, Y., Parrini, M.C., Dulyaninova, N.G., Bresnick, A.R., Mochizuki, N., and Matsuda, M. 2004. Cell type-specific regulation of RhoA activity during cytokinesis. *J. Biol. Chem.* **279**: 44756–44762.
- Zicha, D., Dobbie, I.M., Holt, M.R., Monypenny, J., Soong, D.Y., Gray, C., and Dunn, G.A. 2003. Rapid actin transport during cell protrusion. *Science (Washington, D.C.)*, **300**: 142–145.
- Zurek, B., Sanger, J.M., Sanger, J.W., and Jockusch, B.M. 1990. Differential effects of myosin-antibody complexes on contractile rings and circumferential belts in epitheloid cells. *J. Cell Sci.* **97**(Pt 2): 297–306.