Towards a molecular understanding of cytokinesis

Douglas N. Robinson and James A. Spudich

In this review, we focus on recent discoveries regarding the molecular basis of cleavage furrow positioning and contractile ring assembly and contraction during cytokinesis. However, some of these mechanisms might have different degrees of importance in different organisms. This synthesis attempts to uncover common themes and to reveal potential relationships that might contribute to the biochemical and mechanical aspects of cytokinesis. Because the information about cytokinesis is still fairly rudimentary, our goal is not to present a definitive model but to present testable hypotheses that might lead to a better mechanistic understanding of the process.

During mitosis, a number of events lead up to cytokinesis (Fig. 1). First, the mitotic spindle forms and the chromosomes become aligned at the metaphase plate (Fig. 1, 0:00). During early anaphase, the chromosomes begin to move towards the poles as the spindle elongates. In late anaphase, the spindle has elongated and the cell begins to become more cylindrical (Fig. 1, 1:20). The astral microtubules begin to grow out to the cortex and some of the microtubules are reorganized to extend across the equatorial plane of the cell into the opposite hemisphere. These interzonal microtubules constitute the central spindle once the chromosomes have migrated towards the poles. Several contractile ring proteins, including myosin II, begin to assemble at the equatorial contractile ring (Fig. 1, 3:00). At the end of telophase, the nuclear membrane reforms around the chromosomes in each hemisphere of many cell types. The contractile ring constricts down to the midbody. Finally, the midbody forms an intercellular bridge that connects the two daughter cells and contains mostly spindle and contractile ring components (Fig. 1, 3:00–5:00). Additional proteins are used at this late time to set up the completion of cell division. The midbody is ultimately severed, resulting in full separation of the two daughter cells (Fig. 1, 6:10). By contrast, in some cell types, the midbody is modified into a stable intercellular bridge that allows the cells to remain associated in a syncytium (reviewed in Ref. 1). A summary of the molecules that we will describe can be found in Tables 1–3, and a picture of how some of these molecules might interact is presented in Fig. 2. A more detailed discussion of the various cellular models of cytokinesis can be found in a recent textbook[2]. Other recent comprehensive reviews on cytokinesis and one family of cytokinesis proteins, septins, can also be found[3,4].

The authors are in the Dept of Biochemistry, Beckman Center, Rm B-400, Stanford University, Stanford, CA 94305-5307, USA. E-mail: drobinso@cmgm.stanford.edu

Spatio-temporal control by microtubules

Since the classic experiments of the early twentieth century (reviewed in Ref. 2), the astral microtubules have been implicated in determining the site of cleavage. In a more recent experiment, Rappaport[2] manipulated echinoderm eggs using a glass bead to generate binucleate, horseshoe-shaped cells with a spindle in each arm of the cell. These cells divided between asters of the same spindle and between the asters of adjacent spindles. In recent years, these findings have been extended to other systems, including mammalian cells[5] and the cellular slime mould, *Dictyostelium discoideum*[6].

In mammalian cells, the central spindle is required for the formation of the contractile ring (Refs 7 and 8; reviewed in Ref. 2) and it might be required continuously throughout anaphase and telophase[9]. Recently, it was suggested that in *Drosophila melanogaster* spermatogenesis there might be an interaction between the contractile ring and the central spindle[10]. For example, mutants that are devoid of the mitotic kinesin-like protein, KLP3A, that appears to localize specifically to the central spindle, fail to form a central spindle and a contractile ring.

In addition to KLP3A, other mitotic kinesins have roles in cytokinesis. In mammalian cells, MKLP1 localizes to the nucleus in interphase and then moves to the spindle poles by metaphase of mitosis. By anaphase, it concentrates at the midzone microtubules and is found in the midbody[11]. Mutations in two MKLP1 homologues, the *zen-4* gene in *Caenorhabditis elegans* and the *pavarotti* gene in *D. melanogaster*, lead to defects in cytokinesis[12–14]. In *zen-4* mutants, the cleavage furrow forms and constricts extensively. However, before reaching completion, the cleavage furrow stops contraction and regresses. In *D. melanogaster*, *pavarotti* mutants fail to properly localize anillin, actin and septins to the cleavage furrow. MKLP1 contains two microtubule-binding sites, the ATP-dependent site in the motor domain and an ATP-independent site in the tail, and can bundle microtubules into antiparallel arrays[11]. These proteins might function to crosslink and stabilize the interdigitating microtubules; indeed, in *zen-4* mutant embryos, the central spindle microtubules are absent.

Intriguingly, in binucleate mammalian PtK_1 cells where cleavage furrows form and complete cytokinesis between adjacent spindles, CHO1 (an MKLP1-family protein) associates with the interdigitating microtubules. In cleavage furrows that form between

0962-8924/00/$ – see front matter
PII: S0962-8924(00)01747-5

adjacent spindles but fail to complete cytokinesis, CHO1 does not appear to associate with the interdigitating microtubules[15]. This raises the possibility that MKLP1-family proteins must localize to the interdigitating microtubules to promote successful cytokinesis, regardless of whether the microtubules originate from the central spindle or are associated with astral microtubules. It will be important to identify MKLP1-family proteins in echinoderm eggs and in *D. discoideum* to see whether such molecules associate with the astral microtubules and help to promote cytokinesis, especially because the presence of interdigitating microtubules is not obvious in these systems.

The apparent requirement for interdigitating microtubules for the formation and contraction of the contractile ring suggests that the central spindle sends signals to the cleavage furrow cortex early and late in cytokinesis. In addition to contributing to the assembly of the central spindle, MKLP1-family proteins help to localize the polo kinase, which might transmit part of this signal. The polo kinases have multiple roles in mitosis, including activities in cytokinesis and central spindle assembly (see for example Refs 16 and 17). The polo kinases from mammals and *D. melanogaster* associate with MKLP1 and PAV-KLP in co-immunoprecipitation experiments[13,18]. In *D. melanogaster,* the polo kinase colocalizes with PAV-KLP in the centrosomes and the midbody and becomes mislocalized in *pavarotti* mutants[13]. In *Schizosaccharomyces pombe*, mutations in the polo kinase, Plo1p, do not form a contractile actin ring, disrupting septation. Furthermore, expression of Plo1p in G1 or G2 phase induces ectopic actin rings and septum formation[16]. It is possible, then, that polo kinase is required for the organization of the contractile ring in at least some of these organisms. The fact that the *zen-4* mutants begin contraction whereas the *pavarotti* mutants do not assemble a contractile ring suggests species differences in the exact role that these MKLPs play.

The interdigitating microtubules need to be able to grow for cleavage furrow formation[19], and this ability to grow might be essential for the microtubules to contact the cell cortex where the contractile ring forms[20]. One possible role for growing microtubules in cytokinesis might be to target and stabilize cortical elements, such as the focal adhesions, in the cleavage furrow. Stabilization of focal adhesions in the equatorial cortex of the cell could help to nucleate and anchor the contractile ring actin. Dynamic microtubules have been observed to target interphase focal adhesions, which then capture and stabilize the microtubules[21]. One focal adhesion protein, talin, is required for cytokinesis in *D. discoideum*[22] and is found in the mammalian contractile ring[23]. A second possible role for growing microtubules might be to activate signalling by the Rho-family of small GTPases as dynamic microtubules are necessary for Rac1 (a Rho-family member) activation, leading to lamellipodial formation in interphase fibroblasts[24].

Many of the microtubule signals are regulated temporally by the mitotic cyclin B–p34^{cdc2} kinase complex.

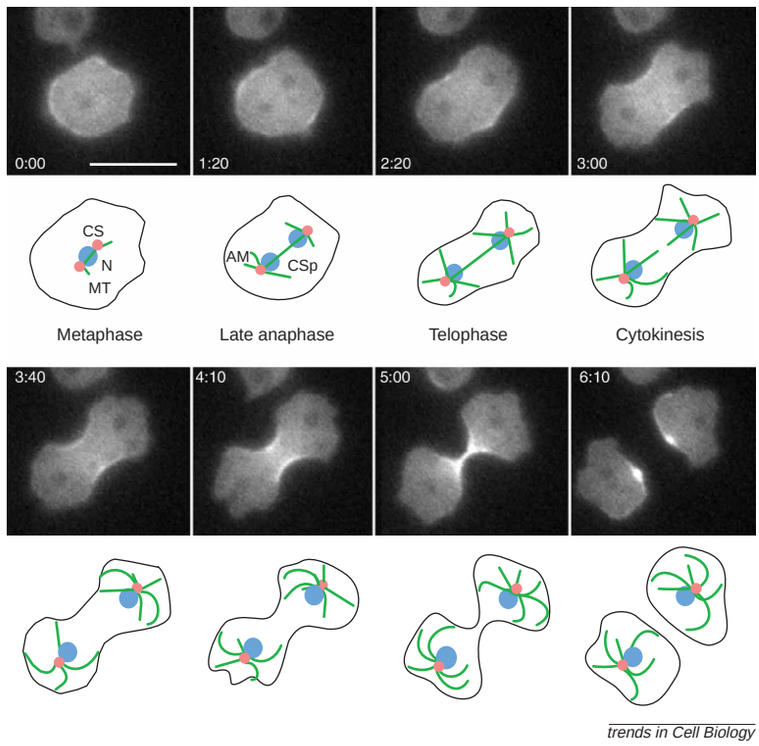

FIGURE 1

Time course for *Dictyostelium discoideum* cytokinesis. At time 0:00, the cell becomes more spherical and quiescent. The green lines represent microtubule (MT) bundles, the blue dot represents the nucleus (N). In *D. discoideum*, the nuclear envelope does not break down completely during mitosis. The pink dots represent the centrosomes (CS). Green-fluorescent protein (GFP)–myosin II is distributed evenly around the cortex. By 1:20, the cell has entered late anaphase and the nuclei appear as separating dark spots, indicating the elongation of the spindle. The MTs extending between the nuclei are bundled antiparallel microtubules that make up the central spindle (CSp). The astral microtubules (AM) are beginning to grow out towards the cell cortex. By 2:20, the cell has reached telophase and the cell becomes more elongated and cylindrical. GFP–myosin II is clearly becoming depleted at the poles and is concentrating along the central midline of the cell. By 3:00, the GFP–myosin II becomes more concentrated at the equator and the cleavage furrow begins to constrict. *D. discoideum* is somewhat different from other organisms in that the central spindle has already begun to disassemble by this time and the interphase MT networks begin to reform. From 3:00 to 5:00, the cell contracts until a residual midbody is formed. By 6:10 the midbody has resolved, separating the two daughter cells. Time is in minutes:seconds. The cell is visualized by monitoring GFP–myosin II expressed in cells mutant for the gene encoding the myosin II heavy chain (*mhcA*)[87]. The MT pattern was schematized according to Kitanishi-Yumura and Fukui[119] and Neujahr *et al.*[6] Bar, 10 μm.

It was thought that cyclin B–p34^{cdc2} kinase phosphorylates myosin II regulatory light chain on its inhibitory sites, thereby blocking cell cleavage until the end of anaphase (reviewed in Ref. 2). However, using sea urchin eggs, Shuster and Burgess[25] provide convincing evidence that cyclin B–p34^{cdc2} kinase does not regulate cytokinesis by inhibiting myosin II activity. Rather, it regulates the formation of the complete elongated spindle with extensive astral microtubules, which does not form until late in anaphase. They further suggest that this regulation ensures the establishment of the proper cell geometry so that the central spindle and/or astral microtubules signal the formation of the cleavage furrow. By mechanically manoeuvring the early anaphase spindle near the cortex, the spindle is able to trigger cleavage furrow contraction[25].

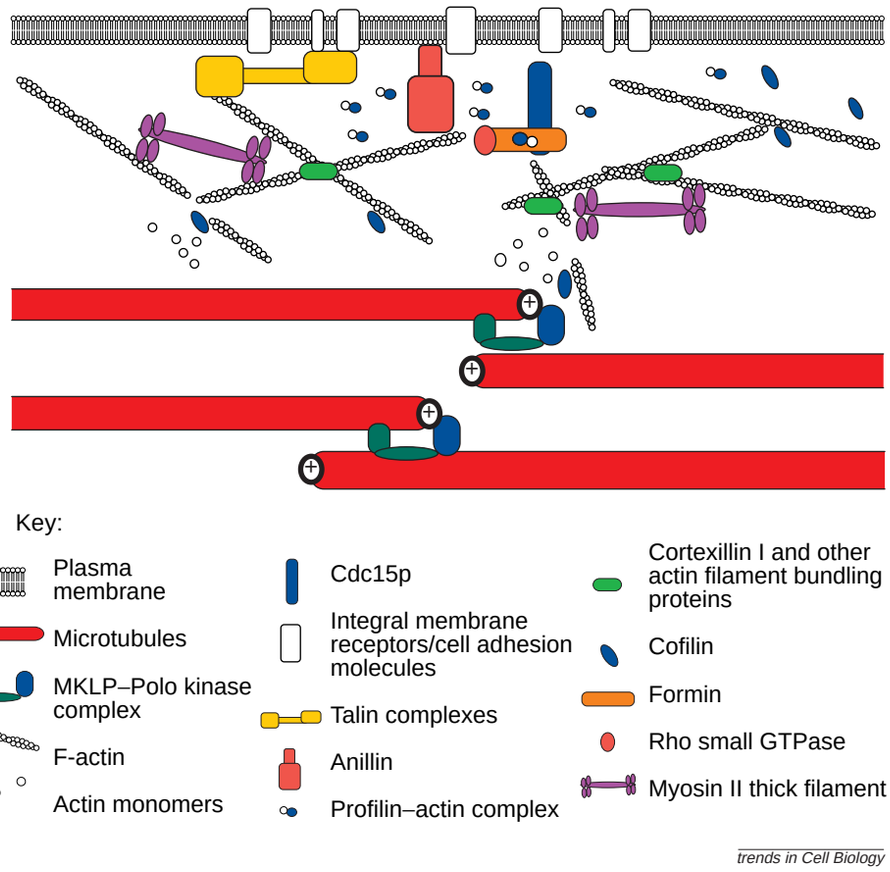

FIGURE 2

A speculative view of the molecular interactions between the central spindle and the cleavage furrow cortex discussed in this review. Although all of the interactions presented might not be universal, each class or type of molecular interaction has been implicated in two or more species. The microtubule-dependent signals might emanate from the interdigitating microtubules. Plus-signs denote the plus-ends of the microtubules, indicating the antiparallel organization. The MKLP-family of mitotic kinesins probably bundles these antiparallel arrays of microtubules, and the associated polo family of kinases might convey this activating signal to the cortex. Talin and anillin might link the contractile ring actin to the plasma membrane. Cdc15p might regulate formins. Formins, profilin and cofilin might regulate the actin polymerization dynamics that contribute to the assembly and disassembly of the contractile ring. Crosslinking proteins such as cortexillin I bundle the actin filaments in the contractile ring. Myosin II and other motor proteins, in addition to actin filament-bundling and depolymerizing proteins, probably contribute to the constriction of the contractile ring.

Other spatio-temporal controllers

Mutagenesis experiments in *S. pombe* have produced several cytokinesis mutants[26]. Three of these, *spg1*, *mid1* and *cdc15*, have phenotypes that indicate they are very important for the spatio-temporal control of the formation of the contractile ring. The Spg1p is a Ras superfamily small GTPase[27] that is localized to the spindle pole body[28]. Loss of this protein leads to a failure in septum formation, whereas overexpression of it can induce septum formation during other times of the cell cycle[27]. *mid1* mutants assemble the contractile ring, but it is often uncentred and/or in an incorrect orientation relative to the plasma membrane (reviewed in Ref. 29) and Mid1p appears to be regulated by the polo kinase, Plo1p (Ref. 30). *cdc15* mutants do not assemble a contractile ring. The Cdc15p protein localizes to the contractile ring and is subject to several levels of regulation[31]. Intriguingly, the *cdc15* transcript is expressed in a cell cycle-dependent manner and increases prior to septal formation. In experiments where Cdc15p is misexpressed at other times in the cell cycle, contractile ring-type structures can be induced to assemble. By contrast, overexpression of Cdc15p results in inhibition of septal formation. Cdc15p protein is also phosphorylated through the cell cycle but is hypophosphorylated during septal formation. These multiple levels of regulation, together with the presence of potential PEST degradation sequences in the protein, indicate the importance of tightly regulating Cdc15p levels. The protein also contains a putative coiled-coil motif and an SH3 domain. A mammalian homologue (PSTPIP) of Cdc15p has been identified that localizes to cleavage furrows and probably has a role in regulating actin dynamics[32]. In *Saccharomyces cerevisiae*, a Cdc15p homologue, Hof1p, interacts directly with a formin-homology protein, Bnr1p[33]. Thus, Cdc15p and PSTPIP might interact directly with formin proteins (discussed below) to initiate the assembly of the contractile ring actin. Clearly, future studies of Cdc15p and PSTPIP will provide great insight into how the assembly of the contractile ring actin is regulated and maintained.

Cortical tension and flow

Most models for animal cytokinesis include a role for the generation of a cortical-tension differential with the peak of tension at the cell equator and the nadir of tension at the cell poles (reviewed in Ref. 2). This cortical-tension differential is thought to contribute to two aspects of cytokinesis: the assembly of the contractile ring by generating cortical flow of contractile ring elements towards the furrow region; and the constriction of the cleavage furrow generated by the actomyosin network.

Cortical flow is thought to be reflected by the migration of cell-surface receptors. Concanavalin A (ConA) receptors and cell-surface beads migrate towards the cleavage furrow during cytokinesis in a variety of cell types[34–36]. The receptors normally diffuse in the plane of the plasma membrane. However, in anaphase when the chromosomes are being pulled towards the poles, ConA receptors between the equator and the position of the chromosomes have vectorial migration towards the centre of the cell with a velocity of the order of 1 μm min^{-1} (Ref. 34). The high velocity and the regional specificity strongly suggest an active mechanism for ConA receptor migration. DeBiasio *et al.*[37] demonstrated a similar type of directional motility of punctate myosin II structures towards the cell equator, whereas myosin II in the polar region showed more random migration.

TABLE 1 – SUMMARY OF PROTEINS: SIGNALLING

	Organism	Location	Discussed role	Refs
Spatiotemporal regulators				
Microtubules (MT)	All organisms	Spindle	Structural, signalling	6–9
Mitotic kinesins	*D. melanogaster* (KLP3A) Mammals (MKLP1) *C. elegans* (Zen-4) *D. melanogaster* (Pavarotti)	Central spindle	MT crosslinking, Polo kinase localization	10–15
Polo kinases	*S. pombe* (plo1) *D. melanogaster* (polo) Mammals (polo)	Spindle poles, central spindle	Signalling	16–18
Cyclin B–p34^{cdc2}	All organisms	Cytosolic	Cell-cycle control	25
Spg1p	*S. pombe*	Spindle poles	Initiation of septum formation	27, 28
Mid1p	*S. pombe*	Nucleus, contractile ring	Determine position and orientation of the contractile ring	30
Cdc15p	*S. pombe* (Cdc15p) Mammals (PSTPIP) *S. cerevisiae* (Hof1p)	Contractile ring Contractile ring Bud neck	Regulate timing, actin dynamics Regulate actin dynamics Interaction with formins	31–33
Small GTPase signalling				
Rho	Mammals, *Xenopus*, *D. melanogaster*	Cortex	Regulate cortical activities	46–48, 53, 71
Pebble/ECT2	Mammals *D. melanogaster*	Central spindle, cleavage furrow	Rho guanine-nucleotide-exchange factor	47, 49
Rho kinase	Mammals	Cleavage furrow	Regulate cytoskeleton	50, 57
Citron kinase	Mammals	Cleavage furrow	Regulate contraction	51
RacE	*D. discoideum*	Global cortex	Regulate cortical tension	39, 42
Myosin light chain phosphatase (myosin-binding subunit)	Mammals	Cleavage furrow	Inhibits myosin II activity, regulated by Rho-kinase	52
Intermediate filaments	Mammals	Extend through cleavage furrow	Structural, regulated by Rho-kinase	50

The causal relationship between the cortical-tension differential and the mechanism of cleavage is not well established. However, examination of mutants in *D. discoideum* suggests that multiple components contribute to the establishment of this differential. Mutations that disrupt myosin II, the RacE small GTPase, or the cortexillin I and II actin-filament crosslinking proteins cause cells to have reduced cortical stiffness[38–40]. The myosin II and RacE mutant cells fail to undergo cytokinesis when the cells are grown in suspension culture[41,42]. Cortexillin I and II single- and double-mutant cells and mutant cells defective for the actin crosslinker coronin have cytokinesis defects when grown on substrates and in suspension culture[43,44]. Coronins belong to a family of actin filament crosslinking proteins that contain a coiled-coil motif and WD40-repeats. RacE and coronin are each globally distributed around the cell, whereas cortexillin I is enriched in the cleavage furrow during cytokinesis.

Cortexillin I encodes a protein that contains a calponin-homology actin-binding domain attached to a coiled-coil motif followed by a 93-amino-acid unique domain that includes a putative phosphatidylinositol (3,4)-bisphosphate [PtdIns(3,4)P_2]-binding site. Cortexillin II has a similar domain structure but lacks the putative PtdIns(3,4)P_2-binding site[44]. Peculiarly, only the C-terminal, 93-residue motif is required for cortexillin I function during cytokinesis[35]. Furthermore, it has been shown that a minimal motif within this C-terminal domain can bundle actin filaments, implying that at least two actin-binding sites exist in this tiny domain[45]. The second of the two actin-binding sites within this motif overlaps with the PtdIns(3,4)P_2-binding site, and PtdIns(3,4)P_2 can compete with actin for association with cortexillin I through this site. Cortexillin I, therefore, might associate with the membrane through the PtdIns(3,4)P_2-binding site and then link actin filaments to the membrane of the cleavage furrow through the remaining actin-binding site of the C-terminal motif and the calponin-homology domain. Alternatively, PtdIns(3,4)P_2 might modulate cortexillin I function in the cleavage furrow. Although a clear homologue of the cortexillins has not been identified in other systems, its conceptual role is probably very important to the process of cytokinesis in most systems.

The differential requirement for myosin II, RacE, coronin and cortexillin I suggests that the mechanisms of generation or maintenance of a cortical-tension gradient during cytokinesis depend on whether the cells can adhere to a surface or are growing in suspension culture. Furthermore, as RacE and coronin are distributed globally during cytokinesis, whereas myosin II and cortexillin I are restricted spatially to the cleavage furrow, cytokinesis can be defined in terms of global stiffness and equatorial contractility-generating systems that lead to cell cleavage.

TABLE 2 – SUMMARY OF PROTEINS: ASSEMBLY AND CONTRACTION

	Organism	Location	Discussed role	Refs
Integral membrane proteins and membrane anchoring				
ConA receptors	Mammals *D. discoideum*	Cleavage furrow	Reflects cortical flow of cytoskeleton	34, 35, 38
CD43	Mammals	Cleavage furrow	Anchor cytoskeleton through radixin	54, 56
Radixin	Mammals	Cleavage furrow	Anchor actin to CD43	55–57
Talin	Mammals *D. discoideum (filopodin)*	Cleavage furrow Unknown	Anchor actin to membrane receptor	22, 23
Anillin	*D. melanogaster*	Cleavage furrow	Anchor cytoskeleton to membrane? Actin filament bundling	58, 60
Contractile ring actin assembly				
Formins	*D. melanogaster* (diaphanous), *S. pombe* (Cdc12p)	Contractile ring	Link Rho signalling to profilin-mediated actin polymerization	53, 68–72
Tropomyosin	*S. pombe* (Cdc8p) Mammals	Contractile ring	Stabilize actin filaments, possibly regulate myosin II	73, 74, 102
Actin filament crosslinking				
Cortexillin I	*D. discoideum*	Contractile ring	Bundle actin filaments, cortical tension	40, 44, 45
Cortexillin II	*D. discoideum*	Unknown		
Coronin	*D. discoideum*	Global cortex	Bundle actin filaments	43
Actin filament dynamics				
Profilin	*D. melanogaster* (chickadee) *D. discoideum*	Global cortex Global cortex	Contractile ring assembly Regulate actin dynamics	63–67
Cofilin	*D. discoideum* Mammals *D. melanogaster* (twinstar)	Global cortex Enriched in furrow Unknown	Actin filament severing Contractile ring disassembly	92–96
Aip1	*S. cerevisiae* (Aip1) *D. discoideum* (DAip1)	Cortical actin Cortical actin	Enhance cofilin activity	93, 94
LIMPII	*D. discoideum* (ImpA)	Vesicles	PtdIns(3,4)P_2 trafficking?	67
Contractile proteins				
Myosin II	All organisms	Contractile ring	Generate contractile force	37, 41, 75–77, 88
Myosin I	Bovine Rat (Myr1)	Cleavage furrow	Possible contractile activity?	89–91
p21-activated kinase	*D. discoideum* (PAKa)	Cleavage furrow	Regulate myosin II localization	82
Caldesmon	Mammals	Part-time at cleavage furrow	Possibly regulate myosin II	103–107

In the 1980s, two models of cytokinesis mechanics were debated. In one view, polar relaxation promotes cell cleavage by relaxing the tension at the poles of the cell. In the opposing view, equatorial stimulation generates tension at the equator through a myosin II-dependent mechanism (reviewed in Ref. 2). Although the myosin II-dependent equatorial contraction model has prevailed, there might in fact be a balance between the global cortical stiffness and the contraction of the equatorial cortex. One prediction, then, is that it might be possible to rescue a mutant partially defective for equatorial contractility by reducing the stiffness of the global cortex. Tests of this hypothesis await a careful genetic analysis of cytokinesis factors involved in the equatorial and global pathways, combined with a detailed physical analysis of the affected cellular mechanics.

Rho-mediated signalling events

During cytokinesis, Rho-mediated signalling is required for the regulation of cortical activities during cytokinesis[46,47] in addition to the recruitment of myosin II and actin filaments to the cleavage furrow[48]. The *D. melanogaster pebble* that encodes an ECT2-family guanine nucleotide exchange factor that is specific for Rho1 is also required for cytokinesis and is localized to the contractile ring similar to anillin (discussed below) and the *Drosophila* peanut (a septin)[47]. *pebble* mutants fail to properly accumulate actin, anillin and peanut in the equatorial region during cytokinesis[47]. A mammalian Rho-nucleotide exchange factor ECT2 localizes to the central spindle and midbody and inhibition of its activity leads to the failure of cytokinesis[49]. Some of the effects of Rho might be carried out by Rho-activated kinases. There is evidence that Rho-activated

citron kinase regulates cleavage furrow contractility[50] and the Rho-kinase regulates separation of the intermediate filaments that extend through the midbody in mammalian cells[51]. Rho-kinase might also regulate contractility by inactivating the myosin II light-chain phosphatase, an inhibitor of myosin II activity, in the cleavage furrow[52]. In addition, some Rho functions are almost certainly carried out by formin proteins[53].

Anchoring the contractile ring to the membrane

Although it is not known exactly how the contractile ring is anchored to the plasma membrane, some proteins that are involved in anchoring the cytoskeleton to the plasma membrane are localized to the cleavage furrow. The CD43 integral membrane protein[54] and radixin[55] localize to the cleavage furrow in mammalian cells. The cytoplasmic domain of CD43 can interact with the N-terminal, globular domain of radixin, that in turn binds to filamentous actin, thereby crosslinking the actin to the plasma membrane[56]. In addition, Rho-kinase phosphorylates radixin, inhibiting radixin's head-to-tail association[57]. This phosphorylation is an important regulatory step for promoting membrane–cortical actin association by radixin and identifies radixin as one more important candidate substrate for Rho-kinase. The focal adhesion protein, talin[23], has also been identified in the cleavage furrow where it might anchor the actin cytoskeleton to integral membrane receptors.

Members of a different family of proteins, the anillins, might also mediate some of the membrane–contractile ring interactions[58]. They contain a pleckstrin-homology domain that is associated with phospholipid binding[59] and can bind and bundle actin filaments. In addition, anillin arrives at the cleavage furrows of meiotic spermatocytes of *D. melanogaster* even in mutant backgrounds such as *chickadee* (profilin) and *KLP3A* that fail to assemble and constrict the actin-rich contractile ring[60].

Assembly of actin-associated proteins

In most systems, a contractile ring contains a band of actin filaments. This band of actin filaments is well organized into antiparallel arrays of microfilaments. Alternatively, in cells such as *D. discoideum*, it is more diffusely distributed along the equatorial zone. Both pre-existing filaments and new actin-filament polymerization contribute to the formation of the contractile ring actin[61,62]. However, genetic studies from a variety of organisms suggest that either of these two mechanisms of actin-filament recruitment are more or less significant depending on the organism. Several genetic experiments in *S. pombe* and *D. melanogaster* have revealed a requirement for the actin-monomer-binding protein, profilin, in cytokinesis[10,63,64]. Profilin has been implicated in regulating actin-polymerization dynamics by sequestering actin monomers, by stimulating nucleotide exchange of actin monomers or by desequestering actin monomers so that they are available for polymerization (reviewed in Ref. 65). The sequestering activity inhibits polymerization whereas the other two activities stimulate polymerization. In *S. pombe* and *D. melanogaster* cells, actin fails to accumulate in the cleavage furrow probably because profilin is needed to stimulate polymerization. By contrast, in *D. discoideum*, cells devoid of profilin[66] can still form a cleavage furrow and myosin II localizes to the cleavage furrow when the cells are grown on surfaces. However, *D. discoideum* does require profilin for cytokinesis in suspension culture. The *D. discoideum profilin* mutant cells overassemble filamentous actin, suggesting that profilin is providing an actin-monomer-sequestering function. Profilin, like many actin-binding proteins, is thought to be regulated by PtdIns(3,4)P_2. Intriguingly, deletion of the *D. discoideum lmpA* gene that encodes LIMPII, a putative PtdIns(3,4)P_2 receptor or transporter, can rescue all of the phenotypes of the *profilin* mutants, providing genetic evidence for PtdIns(3,4)P_2 modulation of cytoskeletal function[67]. However, the apparent phenotypic discrepancy of the *profilin* mutants from different organisms serves to underscore the results of Cao and Wang[61,62] that both pre-existing actin filaments and new actin filament polymerization might contribute to the assembly of the contractile ring actin.

Formins interact with profilin in a variety of genetic and two-hybrid assays[68] and are implicated in the early steps of cytokinesis of *D. melanogaster* (*diaphanous*)[69] and *S. pombe* (*cdc12*)[70]. Mutations in each of these genes leads to failure in contractile ring assembly. Members of this family of proteins are implicated in several actin-based processes and interact with profilin through the proline-rich FH1 domain. Other studies show that Rho and Cdc42 small GTPases can interact directly with a domain of some formins, suggesting that formins might serve as a linker between a signalling cascade and new actin polymerization[53,68,71]. Further evidence of the pivotal role that formins might play in contractile ring assembly comes from *S. pombe*. Overexpressed Cdc12p can be easily detected localizing to a punctate structure over the mitotic nucleus, and, by anaphase, the 'spot' of Cdc12p extends into a ring of protein associated with the new contractile ring. During interphase, the Cdc12p spot colocalizes with and migrates along microtubules[72].

Finally, in *S. pombe*, tropomyosin, an actin-filament binding and stabilizing protein, is required for contractile ring assembly[73]. Loss of tropomyosin, encoded by the *cdc8* gene, leads to a failure to assemble the contractile ring actin, suggesting a role for tropomyosin to stabilize the actin filaments during assembly and/or contraction. In mammalian cells, the tropomyosins have been found in the cleavage furrow[74]. The presence of tropomyosin in the cleavage furrow might also have several implications for the regulation of contraction.

Myosin II has traditionally been thought to localize to the cleavage furrow through its interactions with actin filaments. However, several lines of evidence suggest that myosin II thick filaments might associate with the cleavage furrow through interactions with other proteins. In sea urchin eggs, myosin II remained associated with the cleavage furrow when isolated contractile rings were treated with high salt and ATP to disrupt actin–myosin interactions or the

TABLE 3 – SUMMARY OF PROTEINS: MEMBRANE TRAFFICKING AND COMPLETION

	Organism	Location	Discussed role	Refs
Membrane dynamics				
Syntaxins (t-SNARES)	*A. thaliana* (Knolle1) *D. melanogaster* (syntaxin1) *C. elegans* (Syn-4), sea urchin	Plasma membrane	Exocytosis, membrane insertion	108–111
Clathrin	*D. discoideum*	Membranes	Membrane trafficking	112
LvsA	*D. discoideum*	Unknown	Membrane trafficking	113
Midbody resolution				
Ras	*D. discoideum* (RasG)	Unknown	Signalling	115
IQGAP	*D. discoideum* (GapA)	Unknown	Signalling	116
Calmodulin	*D. discoideum*	Cleavage furrow	Regulatory?	117
Formin	*C. elegans* (cyk-1)	Cleavage furrow	Completion of cytokinesis	118

contractile ring actin was dissolved with gelsolin (reviewed in Ref. 2). In addition, mutant myosin II proteins defective for actin-filament binding still localized to the cleavage furrow in *D. discoideum*[75,76]. In *S. cerevisiae*, the Myo1p type II myosin localized to the bud neck in a septin-dependent, actin-independent manner[77]. Chimeric and truncation studies have indicated that the ability to form myosin II thick filaments is required for localization[78].

D. discoideum myosin II thick filament assembly is regulated by heavy chain phosphorylation. Three threonines in the distal portion of the myosin II rod domain are phosphorylated, causing myosin II thick filaments to disassemble. Substitution of three threonines with aspartic acids mimics the phosphorylated myosin II (3xAsp myosin II), resulting in a myosin II that cannot assemble into thick filaments[79] and does not accumulate at the cleavage furrow[80]. By contrast, substitution of alanines for the three threonines, mimicking the unphosphorylated state, produces a myosin II that forms thick filaments[79] and localizes to the cleavage furrow but does not disassemble, leaving a myosin II aggregate in the midbody[80]. A genetic screen for intragenic suppressors of the 3xAsp myosin II has identified elements of the tail that are likely to participate in the regulatory mechanism[81]. Recruitment of *D. discoideum* myosin II into the cytoskeletal fraction appears to depend on the p21-activated kinase, PAKa[82]. This PAK is also localized to the cleavage furrow. PAK might be inactivating the myosin II heavy chain kinase, thereby indirectly regulating thick filament assembly, or it might be regulating a myosin II thick filament receptor. Studies of heavy chain phosphorylation of mammalian nonmuscle myosin II will undoubtedly shed light on whether this mechanism of myosin II regulation plays a significant role during mammalian cytokinesis.

Cleavage furrow contraction

Nonmuscle myosin II has been classically thought of as the motor protein that drives purse-string cleavage of the cell. It is localized to the cleavage furrows from *S. pombe* to mammals and it is thought to provide the contractile force of the cleavage furrow. Genetic evidence from several organisms, including *D. discoideum* (see for example Refs 41 and 83), *C. elegans*[84] and *S. pombe*[85,86], has suggested a general requirement for myosin II in cytokinesis.

However, in *D. discoideum,* all of the mutant cell lines that are null for myosin II activity still divide on surfaces. In myosin II heavy chain null cells grown on surfaces, these cells form cleavage furrows that are remarkably normal and functional[41,87]. This raises the question of how these cells can generate the forces required to cleave the cell. *S. pombe* has two different myosin II-type molecules, each somewhat unconventional owing to a high proline content in the rod domains, making an extended coiled-coil conformation unlikely[88]. In mammalian cells, two unconventional myosin Is (bovine adrenal medullary myosin I and rat myr1) have been found to localize to the contractile ring[89–91]. The combination of the ability of *D. discoideum* myosin II null cells to divide on substrates and the presence of multiple myosin motors in the cleavage furrows of different organisms raises the possibility that multiple motors contribute to force generation.

In addition to other motors, a combination of actin filament crosslinking proteins and actin depolymerization might contribute to the constriction of the cleavage furrow. The cortexillin I C-terminal domain is sufficient for cortexillin I function in cytokinesis and can crosslink actin filaments. *D. discoideum* mutants defective in the actin-filament-depolymerizing factor, cofilin, are not viable[92]. However, a *D. discoideum* homologue of the yeast Aip1 protein[93], DAip1, is required for normal cytokinesis[94]. The yeast Aip1 interacts genetically with cofilin and enhances the actin filament-severing activity of cofilin[93]. Further evidence supporting a role for cofilin-mediated actin-depolymerization during cytokinesis comes from *D. melanogaster* where *twinstar* (cofilin) mutants fail at cytokinesis[95]. In *twinstar* mutants, actin fails to disassemble from the contractile ring. However, the ring still contracts. Cofilin is also concentrated at the cleavage furrow in mammalian cells[96]. Therefore, constriction of the contractile ring might be accomplished by depolymerizing and severing the actin filaments with cofilin and by progressively bundling the remaining actin filaments with equatorially localized actin filament-crosslinking proteins such as cortexillin I.

Myosin II might also be used at multiple steps during cytokinesis, the first of which might be to help to

establish the proper geometry of the cell during anaphase when the cell elongates. This could help to assemble the contractile ring (Fig. 1, 1:20–2:20). When *D. discoideum* myosin II null cells are grown on substrates, traction forces might allow the cell to elongate, creating the critical geometry that is required for proper assembly. Conversely, when the cells are grown in suspension culture, myosin II might now be essential for this early step of cytokinesis. In the final step of cytokinesis, myosin II, along with additional motors, actin filament-bundling proteins and cofilin-mediated actin depolymerization, might contribute to the constriction of the cleavage furrow. Determination of the extent of contractile ring assembly in the non-adherent myosin II-null cells will help to answer this question.

DeBiasio *et al*.[37] proposed a related model of coupled cortical flow/solation and contraction to describe contraction of the contractile ring. In mammalian cells, they identify myosin II filaments oriented parallel, perpendicular and at angles to the cleavage plane in mammalian cells, and all of these contract during cytokinesis. They also identify myosin II filaments distributed through the lumen of the cleavage furrow, suggesting that myosin II is organized in a gradient of an isotropic array of thick filaments, rather than in a simple discrete purse-string actomyosin ring. Myosin II thick filaments are seen near the poles where they are also contractile, indicating that there are global contractions that occur, and a gradient of myosin II contraction, the peak of which is at the cleavage furrow, might contribute to cell cleavage.

Regulation of the myosin II-mediated contractility

By using a biosensor for the myosin II regulatory light-chain activating phosphorylation on serine-19, DeBiasio *et al*.[37] demonstrated that there is a global rise in phosphorylation of myosin II regulatory light chain by anaphase of mammalian cells. Later, this phosphorylation becomes somewhat spatially restricted to the region of the cleavage furrow by telophase, but this phosphorylation decreases through cytokinesis. Indeed, a requirement for regulatory light chain kinase has been indicated in other systems such as *D. discoideum*[97]. However, a mutant regulatory light chain that cannot be phosphorylated on the activating site rescues the *D. discoideum* regulatory light chain mutant cells[98], suggesting that the regulatory light chain is not the essential substrate for regulatory light chain kinase.

Other components of the actomyosin system are likely to play a crucial regulatory role in the contractile mechanism. In mammalian cells, multiple high- and low-molecular-weight tropomyosin isoforms are encoded by four genes[99]. Some versions of these, such as TM-4 and TM-5, can enhance myosin II actin-activated ATPase activity[100,101]. Tropomyosins, probably TM-4 and TM-5, have recently been demonstrated to localize to the cleavage furrows of mammalian cells where they might help to stimulate myosin II actin-activated ATPase activity[74,102]. Caldesmon, a mammalian Ca^{2+}-regulated protein, is a myosin II heavy chain-, tropomyosin- and actin filament-binding protein[103]. Its binding to actin filaments is antagonized by its phosphorylation by the cyclin B–p34^{cdc2} kinase complex[104,105]. At low molar ratios with actin filaments (1 caldesmon:14–15 actin monomers), caldesmon can inhibit the myosin II ATPase activity. During mitosis, caldesmon is phosphorylated by cyclin B–p34^{cdc2} kinase, rendering it unable to associate with the actin cytoskeleton. After destruction of the cyclin B–p34^{cdc2} kinase in anaphase, caldesmon becomes dephosphorylated and associates with the contractile ring late in cytokinesis[106]. The binding of caldesmon to the cleavage furrow actin filaments might, then, inhibit the myosin II actin-activated ATPase activity[103]. Support for this model comes from the finding that cytokinesis is inhibited by the introduction of a mutant caldesmon that is not phosphorylated as completely by cyclin B–p34^{cdc2} (Ref. 107). Caldesmon's Ca^{2+} sensitivity suggests that it might also be one target of the Ca^{2+} wave that has been observed to accompany the cleavage furrow front in some types of fish cells (reviewed in Ref. 2). The biochemical roles of proteins such as tropomyosin and caldesmon in the cleavage furrow remain to be determined.

Membrane dynamics

To increase the surface area of the cell as it divides, additional membrane might be recruited into the plasma membrane. At least some of this recruitment comes from the insertion of vesicles at the sight of the invaginating cleavage furrow[48]. Syntaxins are t-SNAREs that are required on the target membrane to promote fusion of the vesicle with the target membrane during exocytosis. Recently, mutations in two distantly related syntaxin-encoding genes, the *Arabidopsis thaliana Knolle1*[108] and the *D. melanogaster syntaxin1*[109], have implicated syntaxins in cytokinesis. The *D. melanogaster* syntaxin1 protein is localized to the lateral surfaces of the invaginating membrane during cellularization, and the mutants have large regions of the embryo surface that fail to cellularize. Inhibition of *C. elegans* syntaxin (Syn-4) by RNA interference or the sea urchin syntaxin by antibodies against syntaxin or by the *Botulinum* neurotoxin C1, that proteolyses syntaxins, indicates a requirement for this protein in conventional cytokinesis[110,111].

A role for membrane trafficking has been implicated by *D. discoideum* mutants devoid of either the clathrin heavy chain (chc) protein or the large-volume sphere (LvsA) protein[112,113]. The LvsA protein contains conserved domains found in proteins such as mouse Beige, human Chediak–Higashi protein and human FAN. These homologies point towards a role for LvsA in membrane trafficking or membrane chemistry. Intriguingly, both *chc* and *lvsA* mutant cells form membrane bulges in the cleavage furrow during constriction, leading to failure of cytokinesis[113].

The findings that proteins involved in membrane trafficking are also required for cytokinesis suggest that a careful balance in membrane dynamics is required to ensure proper cell surface remodelling. Raucher and Sheetz[114] recently demonstrated that

endocytosis rates decrease during mitosis, reaching a minimum during metaphase, but then gradually increase again by cytokinesis. The decrease in endocytosis rates correlates with an increase in plasma membrane tension, which undoubtedly has a causal relationship with the cytoskeletal changes occurring as the cell cortex is being remodelled.

Completion of cytokinesis

Several new proteins and signalling events are required for the final severing of the midbody (Fig. 1, 5:00). Loss-of-function mutant *D. discoideum* strains in genes that encode a Ras isoform[115], an IQGAP isoform[116] and calmodulin[117] fail at this late step of cytokinesis. The cells are able to propagate themselves on surfaces because the daughter cells can crawl away from each other until the unsevered midbody finally breaks. In *C. elegans*, the formin protein, cyk-1, appears to be required for a very late step in cytokinesis that occurs after extensive ingression of the furrow[118]. The molecular control of this late step is only beginning to be uncovered and promises to bring many more surprises.

Conclusion

Cytokinesis involves nearly every field of cell biology, including signal transduction, membrane trafficking, integral-membrane and peripheral-cytoskeletal proteins, myosin mechanochemistry and general cellular physiology. Many of the molecular components are being revealed through genetic screens in model systems such as *D. discoideum*, *S. pombe*, *S. cerevisiae*, *C. elegans* and *D. melanogaster*. Although certain themes might not be entirely universal, some mechanisms are emerging as being used widely. These include a requirement for antiparallel-microtubule bundling by kinesin-like proteins. The actin cytoskeletal rearrangements might be regulated by Rho-family small GTPases, profilin and formin-homology proteins. Myosin II motor proteins contribute to the force production that drives cleavage furrow contractility. Finally, the plasma membrane is remodelled and certainly involves syntaxin-mediated exocytosis. The challenges for the future include three major areas. First, the identity and the biochemical properties of all the factors involved must be determined. Second, how each cell type coordinates each of these activities to achieve this carefully orchestrated process must be deciphered. The final challenge will be to determine how each of the molecular pathways relates to the regulatory and mechanical aspects of cell cleavage.

Acknowledgements

We thank Ji-Hong Zang for the GFP–myosin II movie series used to generate Fig. 1. We thank all the members of the Spudich laboratory for numerous useful discussions. This work was supported by a Runyon–Winchell fellowship #DRG1457 to D.N.R. and an NIH grant #GM40509 to J.A.S.

References

1 Robinson, D.N. and Cooley, L. (1996) Stable intercellular bridges in development: the cytoskeleton lining the tunnel. *Trends Cell Biol.* 6, 474–479
2 Rappaport, R. (1996) *Cytokinesis in Animal Cells*, Cambridge University Press
3 Field, C. *et al.* (1999) Cytokinesis in eukaryotes: a mechanistic comparison. *Curr. Opin. Cell Biol.* 11, 68–80
4 Field, C.M. and Kellogg, D. (1999) Septins: cytoskeletal polymers or signalling GTPases? *Trends Cell Biol.* 9, 387–394
5 Sanger, J.M. *et al.* (1998) Unusual cleavage furrows in vertebrate tissue culture cells: insights into the mechanisms of cytokinesis. *Cell Motil. Cytoskeleton* 39, 95–106
6 Neujahr, R. *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
7 Mastronarde, D.N. *et al.* (1993) Interpolar spindle microtubules in PTK cells. *J. Cell Biol.* 123, 1475–1489
8 Cao, L-G. and Wang, Y.L. (1996) Signals from the spindle midzone are required for the stimulation of cytokinesis in cultured epithelial cells. *Mol. Biol. Cell* 7, 225–232
9 Wheatley, S.P. and Wang, Y-L. (1996) Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells. *J. Cell Biol.* 135, 981–989
10 Giansanti, M.G. *et al.* (1998) Cooperative interactions between the central spindle and the contractile ring during *Drosophila* cytokinesis. *Genes Dev.* 12, 396–410
11 Nislow, C. *et al.* (1992) A plus-end-directed motor enzyme that moves antiparallel microtubules *in vitro* localizes to the interzone of mitotic spindles. *Nature* 359, 543–547
12 Raich, W.B. *et al.* (1998) Cytokinesis and midzone microtubule organization in *Caenorhabditis elegans* require the kinesin-like protein zen-4. *Mol. Biol. Cell* 9, 2037–2049
13 Adams, R.R. *et al.* (1998) *pavarotti* encodes a kinesin-like protein required to organize the central spindle and contractile ring for cytokinesis. *Genes Dev.* 12, 1483–1494
14 Powers, J. *et al.* (1998) A nematode kinesin required for cleavage furrow advancement. *Curr. Biol.* 8, 1133–1136
15 Savoian, M.S. *et al.* (1999) Cleavage furrows formed between centrosomes lacking an intervening spindle and chromosomes contain microtubule bundles, INCENP, and CHO1 but not CENP-E. *Mol. Biol. Cell* 10, 297–311
16 Ohkura, H. *et al.* (1995) The conserved *Schizosaccharomyces pombe* kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G_1 and G_2 cells. *Genes Dev.* 9, 1059–1073
17 Carmena, M. *et al.* (1998) *Drosophila* polo kinase is required for cytokinesis. *J. Cell Biol.* 143, 659–671
18 Lee, K.S. *et al.* (1995) Plk is an M-phase specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1. *Mol. Cell. Biol.* 15, 7143–7151
19 Hamaguchi, Y. (1998) Displacement of cleavage plane in the sea urchin egg by locally applied taxol. *Cell Motil. Cytoskeleton* 40, 211–219
20 Wheatley, S.P. *et al.* (1998) Inhibition of chromosomal separation provides insights into cleavage furrow stimulation in cultured epithelial cells. *Mol. Biol. Cell* 9, 2173–2184
21 Kaverina, I. *et al.* (1998) Targeting, capture, and stabilization of microtubules at early focal adhesions. *J. Cell Biol.* 142, 181–190
22 Niewöhner, J. *et al.* (1997) Talin-null cells of *Dictyostelium* are strongly defective in adhesion to particle and substrate surfaces and slightly impaired in cytokinesis. *J. Cell Biol.* 138, 349–361
23 Sanger, J.M. *et al.* (1994) Occurrence of fibers and their association with talin in the cleavage furrows of PtK2 cells. *Cell Motil. Cytoskeleton* 27, 26–40
24 Waterman-Storer, C.M. *et al.* (1999) Microtubule growth activates Rac1 to promote lamellipodial protrusion in fibroblasts. *Nat. Cell Biol.* 1, 45–50
25 Shuster, C.B. and Burgess, D.R. (1999) Parameters that specify the timing of cytokinesis. *J. Cell Biol.* 146, 981–992
26 Chang, F. *et al.* (1996) Isolation and characterization of fission yeast mutants defective in the assembly and placement of the contractile actin ring. *J. Cell Sci.* 109, 131–142
27 Schmidt, S. *et al.* (1997) The Spg1p GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. *Genes Dev.* 11, 1519–1534
28 Sohrmann, M. *et al.* (1998) Asymmetric segregation on spindle poles of the *Schizosaccharomyces pombe* septum-inducing protein kinase Cdc7p. *Genes Dev.* 12, 84–94
29 Chang, F. and Nurse, P. (1996) How fission yeast fission in the middle. *Cell* 84, 191–194
30 Bähler, J. *et al.* (1998) Role of polo kinase and mid1p in determining the site of cell division in fission yeast. *J. Cell Biol.* 143, 1603–1616
31 Fankhauser, C. *et al.* (1995) The *S. pombe cdc15* gene is a key element in the reorganization of F-actin at mitosis. *Cell* 82, 435–444
32 Spencer, S. *et al.* (1997) PSTPIP: a tyrosine phosphorylated cleavage furrow-associated protein that is a substrate for a PEST tyrosine phosphatase. *J. Cell Biol.* 138, 845–860
33 Kamei, T. *et al.* (1998) Interaction of Bnr1p with a novel Src homology 3 domain-containing Hof1p. *J. Biol. Chem.* 273, 28341–28345
34 Koppel, D.E. *et al.* (1982) Surface functions during mitosis. III. Quantitative analysis of ligand-receptor movement into the cleavage furrow: diffusion vs. flow. *J. Cell Biol.* 93, 950–960
35 Weber, I. *et al.* (1999) Cytokinesis mediated through the recruitment of cortexillins into the cleavage furrow. *EMBO J.* 18, 586–594
36 Wang, Y-L. *et al.* (1994) Single particle tracking of surface receptor movement during cell division. *J. Cell Biol.* 127, 963–971
37 DeBiasio, R.L. *et al.* (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
38 Pasternak, C. *et al.* (1989) Capping of surface receptors and concomitant cortical tension are generated by conventional myosin. *Nature* 341, 549–551
39 Gerald, N. *et al.* (1998) A role for *Dictyostelium* RacE in cortical tension and cleavage furrow progression. *J. Cell Biol.* 141, 483–492
40 Simson, R. *et al.* (1998) Membrane bending modulus and adhesion energy of wild-type and mutant cells of *Dictyostelium* lacking talin or cortexillins. *Biophys. J.* 74, 514–522
41 DeLozanne, A. and Spudich, J.A. (1987) Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. *Science* 236, 1086–1091
42 Larochelle, D.A. *et al.* (1996) A novel member of the *rho* family of small GTP-binding proteins is specifically required for cytokinesis. *J. Cell Biol.* 133, 1321–1329
43 deHostas, E.L. *et al.* (1993) *Dictyostelium* mutants lacking the cytoskeletal protein coronin are defective in cytokinesis and cell motility. *J. Cell Biol.* 120, 163–173
44 Faix, J. *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
45 Stock, A. *et al.* (1999) Domain analysis of cortexillin I: actin-bundling, PIP_2-binding and the rescue of cytokinesis. *EMBO J.* 18, 5274–5284

46 O'Connell, C.B. *et al.* (1999) The small GTP-binding protein Rho regulates cortical activities in cultured cells during division. *J. Cell Biol.* 144, 305–313
47 Prokopenko, S.N. *et al.* (1999) A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes Dev.* 13, 2301–2314
48 Drechsel, D.N. *et al.* (1996) A requirement for Rho and Cdc42 during cytokinesis in *Xenopus* embryos. *Curr. Biol.* 7, 12–23
49 Tatsumoto, T. *et al.* (1999) Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G2/M phases, and involved in cytokinesis. *J. Cell Biol.* 147, 921–927
50 Yasui, Y. *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
51 Madaule, P. *et al.* (1998) Role of citron kinase as a target of the small GTPase Rho in cytokinesis. *Nature* 394, 491–494
52 Kawano, Y. *et al.* (1999) Phosphorylation of myosin-binding subunit (MBS) of myosin phosphatase by Rho-kinase *in vivo*. *J. Cell Biol.* 147, 1023–1037
53 Watanabe, N. *et al.* (1997) p140Dia, a mammalian homolog of *Drosophila* diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *EMBO J.* 16, 3044–3056
54 Yonemura, S. *et al.* (1993) Concentration of an integral membrane protein, CD43 (leukosialin, sialophorin) in the cleavage furrow through the interaction of its cytoplasmic domain with actin-based cytoskeletons. *J. Cell Biol.* 120, 437–449
55 Sato, N. *et al.* (1991) Radixin, a barbed end-capping actin-modulating protein, is concentrated at the cleavage furrow during cytokinesis. *J. Cell Biol.* 113, 321–330
56 Yonemura, S. *et al.* (1998) Ezrin/radixin/moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43, and ICAM-2. *J. Cell Biol.* 140, 885–895
57 Matsui, T. *et al.* (1998) Rho-kinase phosphorylates COOH-terminal threonines of Ezrin/Radixin/Moesin (ERM) proteins and regulates their head-to-tail association. *J. Cell Biol.* 140, 647–657
58 Field, C.M. and Alberts, B.M. (1995) Anillin, a contractile ring protein that cycles from the nucleus to the cell cortex. *J. Cell Biol.* 131, 165–178
59 Rebecchi, M.J. and Scarlata, S. (1998) Pleckstrin homology domains: a common fold with diverse functions. *Annu. Rev. Biophys. Biomol. Struct.* 27, 503–528
60 Giansanti, M.G. *et al.* (1999) The role of anillin in meiotic cytokinesis of *Drosophila* males. *J. Cell Sci.* 112, 2323–2334
61 Cao, L-G. and Wang, Y-L. (1990) Mechanisms of the formation of contractile ring in dividing cultured animal cells. I. Recruitment of preexisting actin filaments into the cleavage furrow. *J. Cell Biol.* 110, 1089–1095
62 Cao, L-G. and Wang, Y-L. (1990) Mechanism of the formation of contractile ring in dividing cultured animal cells. II. Cortical movement of microinjected actin filaments. *J. Cell Biol.* 111, 1905–1911
63 Balasubramanian, M.K. *et al.* (1994) The *Schizosaccharomyces pombe cdc3*$^{+}$ gene encodes a profilin essential for cytokinesis. *J. Cell Biol.* 125, 1289–1301
64 Verheyen, E.M. and Cooley, L. (1994) Profilin mutations disrupt multiple actin-dependent processes during *Drosophila* development. *Development* 120, 717–728
65 Theriot, J.A. and Mitchison, T.J. (1993) The three faces of profilin. *Cell* 75, 835–838
66 Haugwitz, M. *et al.* (1994) *Dictyostelium* amoebae that lack G-actin-sequestering profilins show defects in F-actin content, cytokinesis, and development. *Cell* 79, 303–314
67 Karakesisoglou, I. *et al.* (1999) Identification of a suppressor of the *Dictyostelium* profilin-minus phenotype as a CD36/LIMP-II homologue. *J. Cell Biol.* 145, 167–181
68 Evangelista, M. *et al.* (1997) Bni1p, a yeast formin linking cdc42p and the actin cytoskeleton during polarized morphogenesis. *Science* 276, 118–122
69 Castrillon, D.H. and Wasserman, S.A. (1994) *diaphanous* is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the *limb deformity* gene. *Development* 120, 3367–3377
70 Chang, F. *et al.* (1997) cdc12p, a protein required for cytokinesis in fission yeast, is a component of the cell division ring and interacts with profilin. *J. Cell Biol.* 137, 169–182
71 Imamura, H. *et al.* (1997) Bni1p and Bnr1p: downstream targets of the Rho family small G-proteins which interact with profilin and regulate actin cytoskeleton in *Saccharomyces cerevisiae*. *EMBO J.* 16, 2745–2755
72 Chang, F. (1999) Movement of a cytokinesis factor cdc12p to the site of cell division. *Curr. Biol.* 9, 849–852
73 Balasubramanian, M.K. *et al.* (1992) A new tropomyosin essential for cytokinesis in the fission yeast *S. pombe*. *Nature* 360, 84–87
74 Clayton, L. and Johnson, M.H. (1998) Tropomyosin in preimplantation mouse development: identification, expression, and organization during cell division and polarization. *Exp. Cell Res.* 238, 450–464
75 Yumura, S. and Uyeda, T.Q.P. (1997) Transport of myosin II to the equatorial region without its own motor activity in mitotic *Dictyostelium* cells. *Mol. Biol. Cell* 8, 2089–2099
76 Zang, J-H. and Spudich, J.A. (1998) Myosin II localization during cytokinesis occurs by a mechanism that does not require its motor domain. *Proc. Natl. Acad. Sci. U. S. A.* 95, 13652–13657
77 Bi, E. *et al.* (1998) Involvement of an actomyosin contractile ring in *Saccharomyces cerevisiae* cytokinesis. *J. Cell Biol.* 142, 1301–1312
78 Shu, S. *et al.* (1999) Role of myosin II tail sequences in its function and localization at the cleavage furrow in *Dictyostelium*. *J. Cell Sci.* 112, 2195–2201
79 Egelhoff, T.T. *et al.* (1993) *Dictyostelium* myosin heavy chain phosphorylation sites regulate myosin filament assembly and localization *in vivo*. *Cell* 75, 363–371
80 Sabry, J.H. *et al.* (1997) Myosin heavy chain phosphorylation sites regulate myosin localization during cytokinesis in live cells. *Mol. Biol. Cell* 8, 2647–2657
81 Liang, W. *et al.* (1999) A structural model for phosphorylation control of *Dictyostelium* myosin II thick filament assembly. *J. Cell Biol.* 147, 1023–1038
82 Chung, C.Y. and Firtel, R.A. (1999) PAKa, a putative PAK family member, is required for cytokinesis and the regulation of the cytoskeleton in *Dictyostelium discoideum* cells during chemotaxis. *J. Cell Biol.* 147, 559–575
83 Chen, P. *et al.* (1994) Targeted disruption of the *Dictyostelium* RMLC gene produces cells defective in cytokinesis and development. *J. Cell Biol.* 127, 1933–1944
84 Shelton, C.A. *et al.* (1999) The nonmuscle myosin regulatory light chain gene *mlc-4* is required for cytokinesis, anterior–posterior polarity, and body morphology during *Caenorhabditis elegans* embryogenesis. *J. Cell Biol.* 146, 439–451
85 Kitayama, C. *et al.* (1997) Type II myosin heavy chain encoded by the *myo2* gene composes the contractile ring during cytokinesis in *Schizosaccharomyces pombe*. *J. Cell Biol.* 137, 1309–1319
86 Bezanilla, M. *et al.* (1997) Identification of a second myosin-II in *Schizosaccharomyces pombe*: Myp2p is conditionally required for cytokinesis. *Mol. Biol. Cell* 8, 2693–2705
87 Zang, J-H. *et al.* (1997) On the role of myosin-II in cytokinesis: division of *Dictyostelium* cells under adhesive and nonadhesive conditions. *Mol. Biol. Cell* 8, 2617–2629
88 May, K.M. *et al.* (1997) Type II myosin involved in cytokinesis in the fission yeast, *Schizosaccharomyces pombe*. *Cell Motil. Cytoskeleton* 38, 385–396
89 Wagner, M.C. *et al.* (1992) Tissue distribution and subcellular localization of mammalian myosin I. *J. Cell Biol.* 119, 163–170
90 Breckler, J. and Burnside, B. (1994) Myosin I localizes to the midbody region during mammalian cytokinesis. *Cell Motil. Cytoskeleton* 29, 312–320
91 Ruppert, C. *et al.* (1995) Localization of the rat myosin I molecules myr 1 and myr 2 and *in vivo* targeting of their tail domains. *J. Cell Sci.* 108, 3775–3786
92 Aizawa, H. *et al.* (1995) Identification, characterization, and intracellular distribution of cofilin in *Dictyostelium discoideum*. *J. Biol. Chem.* 270, 10923–10932
93 Rodal, A.A. *et al.* (1999) Aip1p interacts with cofilin to disassemble actin filaments. *J. Cell Biol.* 145, 1251–1264
94 Konzok, A. *et al.* (1999) DAip1, a *Dictyostelium* homologue of the yeast actin-interacting protein 1, is involved in endocytosis, cytokinesis, and motility. *J. Cell Biol.* 146, 453–464
95 Gunsalus, K.C. *et al.* (1995) Mutations in *twinstar*, a *Drosophila* gene encoding a cofilin/ADF homologue, results in defects in centrosome migration and cytokinesis. *J. Cell Biol.* 131, 1243–1259
96 Nagaoka, R. *et al.* (1995) Concentration of cofilin, a small actin-binding protein, at the cleavage furrow during cytokinesis. *Cell Motil. Cytoskeleton* 30, 1–7
97 Smith, J.L. *et al.* (1996) Myosin light chain kinase (MLCK) gene disruption in *Dictyostelium*: a role for MLCK-A in cytokinesis and evidence for multiple MLCKs. *Proc. Natl. Acad. Sci. U. S. A.* 93, 12321–12326
98 Ostrow, B.D. *et al.* (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
99 Pittenger, M.F. *et al.* (1994) Functional properties of non-muscle tropomyosin isoforms. *Curr. Opin. Cell Biol.* 6, 96–104
100 Novy, R.E. *et al.* (1993) *In vitro* functional characterization of bacterially expressed human fibroblast tropomyosin isoforms and their chimeric mutants. *Cell Motil. Cytoskeleton* 26, 248–261
101 Fanning, A.S. *et al.* (1994) Differential regulation of skeletal muscle myosin-II and brush border myosin-I enzymology and mechanochemistry by bacterially produced tropomyosin isoforms. *Cell Motil. Cytoskeleton* 29, 29–45
102 Berthier, C. and Helfman, D.M. (1998) Dynamic distribution of tropomyosin isoforms in fibroblasts. *Mol. Biol. Cell* 9, 135a
103 Marston, S.B. and Redwood, C.S. (1991) Review article: the molecular anatomy of caldesmon. *Biochem. J.* 279, 1–16
104 Yamashiro, S. *et al.* (1991) Phosphorylation of non-muscle caldesmon by p34^{cdc2} kinase during mitosis. *Nature* 349, 169–172
105 Yamashiro, S. *et al.* (1990) Mitosis-specific phosphorylation causes 83K non-muscle caldesmon to dissociate from microfilaments. *Nature* 344, 675–678
106 Hosoya, N. *et al.* (1993) Localization of caldesmon and its dephosphorylation during cell division. *J. Cell Biol.* 121, 1075–1082
107 Warren, K.S. *et al.* (1996) Overexpression of microfilament-stabilizing human caldesmon fragment, CaD39, affects cell attachment, spreading, and cytokinesis. *Cell Motil. Cytoskeleton* 34, 215–229
108 Lauber, M.H. *et al.* (1997) The *Arabidopsis* KNOLLE protein is a cytokinesis-specific syntaxin. *J. Cell Biol.* 139, 1485–1493
109 Burgess, R.W. *et al.* (1997) The synaptic protein syntaxin1 is required for cellularization of *Drosophila* embryos. *J. Cell Biol.* 138, 861–875
110 Conner, S.D. and Wessel, G.M. (1999) Syntaxin is required for cell division. *Mol. Biol. Cell* 10, 2735–2743
111 Jantsch-Plunger, V. and Glotzer, M. (1999) Depletion of syntaxins in the early *Caenorhabditis elegans* embryo reveals a role for membrane fusion events in cytokinesis. *Curr. Biol.* 9, 738–745
112 Niswonger, M.L. and O'Halloran, T.J. (1997) A novel role for clathrin in cytokinesis. *Proc. Natl. Acad. Sci. U. S. A.* 94, 8575–8578
113 Kwak, E. *et al.* (1999) LvsA, a protein related to the mouse beige protein, is required for cytokinesis in *Dictyostelium*. *Mol. Biol. Cell* 10, 4429–4439
114 Raucher, D. and Sheetz, M.P. (1999) Membrane expansion increases endocytosis rate during mitosis. *J. Cell Biol.* 144, 497–506
115 Tuxworth, R.I. *et al.* (1997) *Dictyostelium* RasG is required for normal motility and cytokinesis, but not growth. *J. Cell Biol.* 138, 605–614
116 Adachi, H. *et al.* (1997) *Dictyostelium* IQGAP-related protein specifically involved in the completion of cytokinesis. *J. Cell Biol.* 137, 891–898
117 Liu, T. *et al.* (1992) Inducible expression of calmodulin antisense RNA in *Dictyostelium* cells inhibits the completion of cytokinesis. *Mol. Biol. Cell* 3, 1403–1413
118 Swan, K.A. *et al.* (1998) Cyk-1: a *C. elegans* FH gene required for a late step in embryonic cytokinesis. *J. Cell Sci.* 111, 2017–2027
119 Kitanishi-Yumura, T. and Fukui, Y. (1987) Reorganization of microtubules during mitosis in *Dictyostelium*: dissociation from MTOC and selective assembly/disassembly in situ. *Cell Motil. Cytoskeleton* 8, 106–117