New horizons for cytokinesis

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The mechanism of cytokinesis is an old problem in cell biology that has received fresh attention recently with a large variety of powerful approaches and experimental systems. Significant advances have been made on the structure of the cortical cytoskeleton, the identification of proteins and genes involved, and the regulatory mechanism. Many surprises have surfaced within the past two years, leading us toward a major revision in our understanding of this important process.

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Introduction

Over the past few years a strong resurgence in studies of cell division has been fueled by intense interest in defining the molecular mechanisms regulating the cell cycle, chromosome segregation, and cell cleavage. Expansion of a century's worth of knowledge on cytokinesis has come from a variety of approaches. Methods of micromanipulation and advanced microscopy continue to provide important clues to the dynamics and mechanics of cell cleavage, while advances in our knowledge of the molecular genetics of flies, yeast, fungi, and slime molds have resulted in a rapid extension of the list of proteins involved in various aspects, from signal transduction to mechanotransduction. These powerful tools have brought new insights that thrust us toward a more comprehensive understanding of cell cleavage at the molecular level.

This review evaluates only the most recent findings that are likely to challenge our understanding of cell division in animal cells. For more comprehensive reviews of past literature and of cell division in plants, readers are directed to a host of excellent books and articles [1–8].

Organization and dynamic remodeling of the cortex during cell cleavage

The 'purse string' model for cytokinesis, based on the muscle contraction paradigm, proposes that cell cleavage occurs by the contraction of a circumferential ring of actin and myosin II at the cell equator (Fig. 1a). This model still stands as the mainstream explanation for cell division, largely because of its ability to account for a wide variety of observations. Some recent studies, however, have begun to challenge the basic tenets of the purse string model.

One serious limitation in modeling cell cleavage has arisen from the difficulty of obtaining detailed information on the organization of actin and myosin in dividing cells in relation to the progress of cell cleavage and the development of tension. Mabuchi [9•] recently obtained improved images of actin filaments in dividing sea urchin eggs attached to protamine-coated slides. It appeared that during the course of cleavage, actin gradually reorganized from short, randomly oriented filaments into striking equatorial bundles. Maupin *et al.* [10•] improved the capacity to resolve myosin organization in dividing HeLa cells by using a combination of direct immunofluorescence and confocal scanning microscopy. What appeared previously as uniform staining in the furrow was revealed as numerous discrete spots, each possibly corresponding to a small group of myosin minifilaments [11]. A number of proteins, including filamin and talin, show a similar punctate appearance following the microinjection of their fluorescent analogs into well spread epithelial cells during division [12•]. From the apparent linear arrangement of such puncate structures, Sanger *et al.* [12•] suggested that cleavage might be achieved through the shortening of structures similar to stress fibers, as in the contraction of stress fibers in interphase cells.

We had recently raised the possibility [13••], however, that equatorial bundles of actin filaments may not be a prerequisite for cell division. We examined actin organization in a strongly adhesive subclone of normal rat kidney cells with a combination of three-dimensional image reconstruction and fluorescence-detected linear dichroism microscopy. Interestingly, the dorsal cortex, which undergoes active cleavage, showed only poorly organized actin filaments, whereas the ventral cortex, which undergoes little cleavage because of the resistance from adhesion to the substrate and neighboring cells, developed well organized bundles during the course of cytokinesis. These observations suggested that equatorial orientation of actin filaments may represent a consequence of contraction and resistive forces, rather

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Abbreviations

$[Ca^{2+}]_i$—intracellular Ca^{2+} concentration; **MLC**—myosin light chain; **MLC_{20}**—20 kDa myosin light chain; **MHC**—myosin heavy chain.

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than being a requirement or common denominator of all dividing cells. The possible alternative mechanisms of cytokinesis are illustrated in Fig. 1.

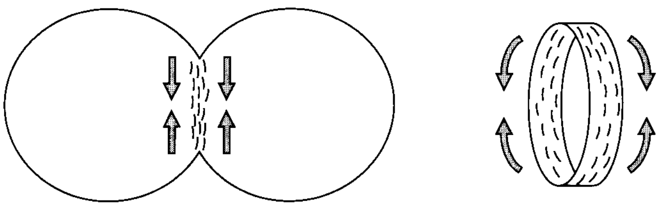
In addition to equatorial filaments, a second set of actin filaments was found to associate end-on with the membrane, and a third set to extend along the spindle axis in the region flanking the equator [13••]. The latter can be detected during the earliest stage of cytokinesis, and probably corresponds to filaments being recruited into the cleavage furrow, as seen in previous studies. This transport process apparently involves a whole set of membrane and cortical elements, including membrane proteins that bind concanavalin A, radixin (a member of the ezrin superfamily), and a number of actin-binding proteins such as filamin and α-actinin. Recent additions to the list of membrane receptors that colocalize with actin include wheat germ agglutinin binding sites [9•], integrins [14•], and CD43 [15•]. In addition, Yonemura *et al.* [15•] showed that the transport of CD43 to the cleavage furrow requires its cytoplasmic ezrin-binding domain. Although the transport of actin filaments may represent a crucial step for the assembly of contractile structures, the coupled transport of a broad range of membrane proteins is also likely to play important roles in the anchorage of actin filaments, and in the localization of molecules involved in signaling pathways.

A role for other cytoskeletal proteins in cytokinesis?

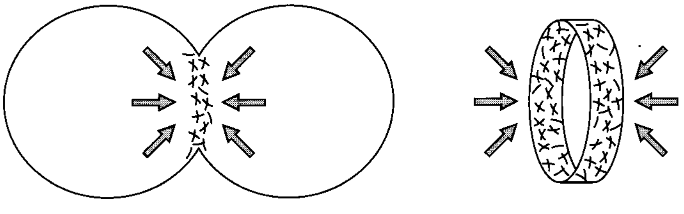
New studies have provided evidence for the involvement of a number of cytoskeletal proteins in cytokinesis. Many of them, including tropomyosin [16], caldesmon [17•], calmodulin [18,19•] and unconventional myosins [20,21••], appear to provide positive or negative regulation of the mechanical properties of the actin-rich cortex. For example, the localization of tropomyosin at the site of septation is thought to help stabilize actin filaments during ring formation in yeast [16], and decreased amounts of cortical caldesmon may lead to decreased actin filament cross-linking and increased actin–myosin interactions in dividing tissue culture cells [17•].

Other proteins, such as cofilin [22], profilin [23•], and coronin [24•], may regulate cytokinesis by modulating the polymerization state of actin. In studies with fission yeast profilin (cdc3) mutants, Balasubramanian *et al.* [23•] provided direct evidence that profilin is essential for cytokinesis and appears to be involved in the assembly of the F-actin ring during septation. Overloading tissue culture cells with profilin by microinjection does not seem to cause any visible effects during cytokinesis, but does cause disassembly of stress fibers in interphase cells [25]. Together, these studies suggest that profilin may

(a) Purse string



(b) Isotropic contraction



(c) Traction

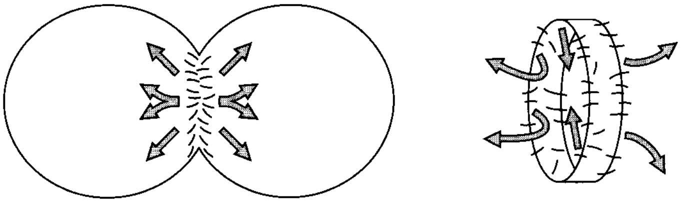


Fig. 1. Possible models of force generation and transduction during cytokinesis. Actin filaments responsible for transmitting forces are depicted as lines. Arrows indicate the direction of forces. Panels on the right show a three-dimensional view of the furrow region. In the conventional purse string model (**a**), cleavage is achieved by a circumferential ring of actin and myosin filaments. Myosin filaments interact with antiparallel actin filaments along the equator, causing them to slide against one another and to shrink in diameter. In the isotropic contraction model (**b**), cleavage is achieved through a random network of actin and myosin filaments in the equatorial region. Sliding of actin filaments within such a random network would cause the equatorial region to shrink along all directions [13••]. In the traction model (**c**), cleavage is achieved by the pulling forces of actin filaments associated end-on with the membrane. Forces exerted by filaments extending toward the cytoplasm would cause the membrane to sink inward. Forces from filaments extending toward the opposite poles would split the equatorial region into daughter cells. It is likely that multiple mechanisms coexist and cooperate during cleavage.

play an important role in establishing a proper balance of actin assembly and disassembly in dividing cells, and suggest that different subsets of actin filaments (such as those in the equator) can exhibit different susceptibility to profilin-induced disassembly. In addition to profilin, coronin-like proteins have also been shown to affect actin assembly in dividing *Dictyostelium* amoebae [24•]. The restricted localization of coronin in the polar region of dividing cells suggests that it may be involved in generating traction forces in separating daughter cells [24•,26•].

The use of molecular genetics and new screening approaches in systems once thought to display unconventional modes of cell cleavage, including *Drosophila* embryos, budding and fission yeast, fungi, and bacteria, is providing a powerful means for defining new molecular components in cell division [27••,28••,29,30]. Mutations in the fly that disrupt such distinct processes as nuclear movements, nuclear organization, and surface morphology during embryogenesis have revealed genes (with names such as *grapes, scrambled, nuclear fallout, sponge, pebble, nullo,* and *bottleneck*) that appear to encode proteins essential for various aspects of pseudocleavage, cellularization, and cytokinesis [27••,31•,32••]. In addition, new screening methods have been developed that greatly facilitate the detection of genes involved in cleavage. One novel method, for example, involves expressing libraries of fly genes in yeast and screening for phenotypes that affect cell shape changes during growth and division [33•].

Although the function of most of these proteins is still undefined, the defective cytoskeletal phenotypes of their mutants suggest that they may hold important clues to various mechanisms, from actin-dependent force generation to microtubule-dependent regulation. One particularly enlightening finding is the discovery of the *Drosophila peanut* gene [34••], an apparent relative of four *Saccharomyces cerevisiae* cell cycle genes whose gene products make up the 10 nm neck filaments found at the budding site [35•,36]. Interestingly, mutations in the *peanut* gene cause severe defects in cytokinesis of imaginal tissues, with formation of large multinucleated cells [34••]. Moreover, localization of wild-type peanut protein shows strong staining in the cleavage furrow of dividing cells, intercellular bridges, and advancing furrow canals and rings of cellularizing embryos. Although it is still unclear whether peanut forms 10 nm neck filaments in fly, its simultaneous localization with actin during furrowing and its presence in the intercellular bridges that persist between daughter cells (midbodies) suggests that it may affect the determination of the site of cell cleavage and maintenance of the midbody [34••]. In addition, this family of proteins appears to contain conserved p-loop motifs that are involved in GTP/ATP binding [34••,35•,36], and that are similar to those in the GTP-dependent filament-forming ftsZ protein that assembles at the site of septation in *Escherichia coli* [37,38•]. These findings suggest that a new class of evolutionarily conserved, nucleotide-dependent filament systems, similar to those of actin and tubulin, may be involved in cell division. Given the apparent homology that the yeast and fly proteins share with a mouse homologue [34••,36], it will be of great interest to see if similar proteins function in higher vertebrate cell division, especially in response to the ras/rho GTP-dependent signaling pathways involved in regulating cell division [39•,40].

Signaling during cytokinesis

The dynamics of cell cleavage are defined by complex chemical and mechanical signals. Previous studies have addressed both initial signaling events, such as changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) [41], and downstream events that regulate specific protein functions, such as phosphorylation and dephosphorylation [6].

Intracellular calcium

Recent experiments continue to strengthen the idea that a localized, transient rise in $[Ca^{2+}]_i$ at anaphase onset plays an important role in regulating cytokinesis in early embryos [42•–45•]. These oscillatory transients are tightly tied to the rhythm of the cell cycle clock established during egg activation [43•], and appear to correlate with cyclic increases in inositol trisphosphate levels [42•]. Genetic and biochemical studies have started to shed some light on the mechanism of such changes in second messenger levels. For example, observations with yeast mutants suggest that key roles are played by a variety of genes involved in signal transduction, including those encoding phosphatidylinositol 4-kinase [46•], protein kinase C [47], GTP-binding proteins [40,48•], and proteins containing Src homology region 3 domains [49]. In addition, mutations in numerous other genes involved in regulating cytoskeletal functions [50•,51•], metabolism [52,53], cell cycle control [54•,55–57], and phosphatase activities [58,59] also result in cytokinetic defects. These studies suggest that the path to normal cell division is paved by complex biochemical circuits that govern the dynamic interplay between signaling and mechanotransduction pathways.

Phosphorylation/dephosphorylation

The idea of a direct role of protein phosphorylation in regulating contractility during cell division gained new ground two years ago, when Satterwhite *et al.* [60] showed that the cell-cycle dependent $p34^{cdc2}$ kinase could phosphorylate the negative regulatory sites (Ser1, 2 and 9) of the 20 kDa myosin light chain protein (MLC_{20}) *in vitro*. Thus, myosin filament assembly and activity might be suppressed during metaphase when $p34^{cdc2}$ activity is high, and reactivated upon anaphase onset with the decrease in $p34^{cdc2}$ activities. These studies were recently expanded by Yamakita *et al.* [61••], who showed that the level and sites of MLC_{20} phosphorylation undergo a dramatic change during mitosis in dividing tissue culture cells. The site of phosphorylation switches from the negative regulatory sites Ser1 and Ser2 early in cell division to the stimulatory Ser19 site later in cytokinesis, consistent with the idea that the phosphorylation of MLC_{20} might regulate cytokinesis. Given the apparently tight regulation of cell cleavage by $[Ca^{2+}]_i$ [44•,45•], it seems particularly attractive that Ca^{2+}/calmodulin dependent kinases, such as the myosin light chain kinase, regulate (at least in part) actin–myosin contractility [62].

The role of myosin light chains (MLCs) in cytokinesis may be much more complex than was originally proposed by Satterwhite *et al.* [60]. On the one hand, the importance of MLCs during cell division has received support from several recent studies employing gene manipulation techniques. Gene disruptions in the regulatory MLC of *Drosophila spaghetti squash* embryos [63] and in either the essential or regulatory MLC of *Dictyostelium* amoebae caused severe defects in cytokinesis [64•]. On the other hand, in a surprising study Uyeda and Spudich [65••] showed that cytokinesis is not significantly affected when regulatory MLCs are removed from whole myosin molecules by deleting the MLC-binding site in the neck region of the heavy chain. From these results, they concluded that regulatory MLC interactions were not essential for myosin mechanotransduction or regulation during cell division. They further speculated that MLC gene disruption probably causes defects by increasing the *in vivo* aggregation of myosin heavy chains (due to the exposure of an α-helical neck region). The observations of Uyeda and Spudich [65••] appear to be consistent with the recent findings in *Dictyostelium* showing that the phenotype of MLC-null cells can be rescued by expression of mutant MLC constructs carrying an alanine replacement at the stimulatory phosphorylation site [66••]. In contrast, a similar replacement in the *Drosophila* MLC is critical for *in vivo* functions during embryogenesis, whereas alanine replacements of the putative $p34^{cdc2}$ phosphorylation sites appear to have no effect on cytokinesis (R Karess, S Wheatley, P Jordan, S Kulkarni: abstract in *Mol Biol Cell* 1993, 4:260a). Thus the questions remain: are there selective advantages in having MLC regulatory mechanisms in dividing cells? Do MLCs play any role in fine-tuning force production during cleavage? Are MLCs involved in other cellular functions?

In addition to regulation through MLCs, several new studies point to the potential importance of myosin heavy chain (MHC) regulation during cell cleavage. Larochelle and Epel [67•] showed that MHC phosphorylation apparently decreases during the first cleavage of sea urchin embryos, and that a significant amount of dephosphorylated MHC is present in isolated cleavage furrows. Moreover, Egelhoff *et al.* [68••] used site-directed mutagenesis to convert three threonine residues at phosphorylation sites in the carboxy-terminal 'assembly region' of *Dictyostelium* MHC to either alanine residues or to aspartic acid residues (which mimic phosphorylated threonine). In transfected cells, alanine mutants were capable of cell division even in the presence of an overabundance of assembled myosin, whereas negatively charged aspartic acid mutants failed to divide. Taken together, these data suggest that the dephosphorylation of MHCs may play an important role in the assembly of myosin filaments in the cleavage furrow.

In addition to myosin, other cytoskeletal proteins such as caldesmon and spectrin are also phosphorylated during cell division [17•,69]. Matsumura and colleagues [17•] showed that $p34^{cdc2}$-dependent phosphorylation of caldesmon causes a decrease in its association with the actin cytoskeleton during prophase. Upon exit from mitosis, decreases in caldesmon phosphorylation occur concomitantly with an increased association with newly formed actin stress fibers. The involvement of phosphorylation is likely to go beyond myosin, caldesmon, and spectrin, and it will be a challenging task to map out the exact regulatory circuitry that governs structural assembly and contractility in dividing cells.

The role of microtubules in cytokinesis

Successful cell division requires a tight spatial and temporal coordination between mitosis and cytokinesis. Several new observations strengthen the notion that mitotic spindles can somehow regulate cortical contractility. Rappaport and Rappaport [70] refined their earlier observations [4] and showed that cleavage can be induced over a prolonged period of time throughout the cortex, as long as it is within a short distance of the spindle. Hird and White [71••] examined cortical dynamics in dividing *Caenorhabditis elegans* embryos by following the movement of cortical organelles. When the spindle was dislocated near the cortex by drug treatment, the dislocation induced profound changes in the pattern of cortical transport and parallel distortions in cortical actin organization.

Clearly, microtubules represent the most plausible candidates for mediating the coordination between the spindle and the cortex. The role of microtubules is further demonstrated in our recent study (DJ Fishkind, JD Silverman, Y-L Wang, unpublished data), showing that cortical movement and actin organization in dividing cells are highly sensitive to the application of the microtubule-targeting drugs nocodazole and taxol. What remains largely unknown, however, is the nature of the molecular mechanism involved in microtubule–cortical interactions. Until recently, most studies have emphasized the importance of spindle asters and astral microtubules ([4]; see also [72•] for a recent review), rather than chromosomes or the spindle interzone (the central region of the anaphase spindle between separating chromosomes). Although little is known at the molecular level, the possible role of asters and astral microtubules is supported by some compelling observations such as the appearance of a cleavage furrow between each pair of asters in eggs with multiple spindles [4,72•].

Recent studies, however, point to the potential importance of the spindle interzone. For example, Wang *et al.* [14•] examined the transport of fluorescent latex particles bound to the surface of dividing cells. The particles (and associated cortical components) moved toward the cleavage furrow ~1 min after the onset of anaphase, with a pattern closely correlated with the development of the spindle interzone. Miller *et al.* [44•] noticed that when the position of cell cleavage is perturbed by the injection of calcium buffers, the pattern of furrowing appears to be dictated by the position of the spindle interzone rather than the poles. The idea of the involvement of the spindle interzone is appealing from the point of view of regulation, as it would ensure a tight spatial and temporal coordination between chromosome separation and cytokinesis.

Of particular interest is the recent discovery of an increasing number of microtubule-associated proteins concentrated in the spindle interzone during late anaphase and telophase (reviewed by Margolis and Andreassen [73]; see also [74–75] for descriptions of new members of this group of proteins). Included in this group are γ-tubulin, which may be involved in microtubule nucleation [76•], and several kinesin-like motor proteins, such as the CHO1 antigen and CENP-E, which may be responsible for transporting other components to the spindle interzone. These proteins appear to form a discrete disc-like structure in the cleavage furrow [73–75], and they have been implicated as affecting cytokinesis by several recent experiments. For example, microinjection of CHO1 antibodies can cause inhibition of cytokinesis [77•], and CENP-E activity peaks during a short window of time between the onset of anaphase and the entry into G_1 phase, exactly when cytokinesis takes place [78•,79•]. Although these observations are suggestive, more evidence is required to demonstrate the direct involvement of interzonal microtubules and their associated proteins in cytokinesis.

Microtubules may modulate cortical activities through chemical signaling or physical interactions. Successful modeling of cleavage has been achieved by assuming that microtubules mediate the transport of signaling molecules to the cortex [80•,81]. Moreover, a recent

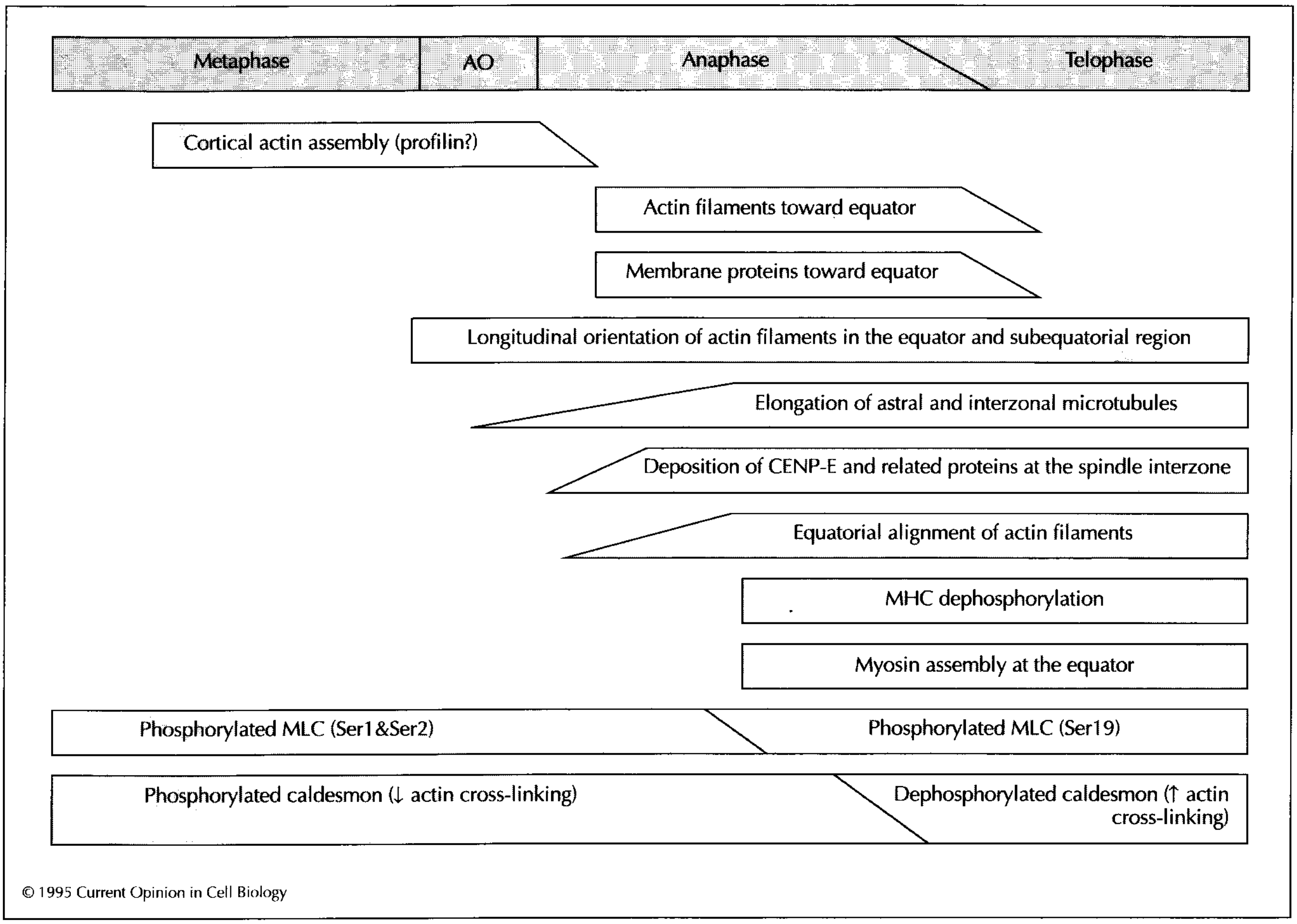


Fig. 2. Temporal organization of major cytoskeletal events related to cytokinesis. These include actin dynamics, membrane and cortical restructuring, development of the spindle midzone, and changes in the phosphorylation state of caldesmon and myosin. Assembly of cortical actin and increases in phosphorylation of caldesmon and of the 20kDa myosin light chain (MLC) at Ser1 and Ser2 precede the movement of F-actin and receptors to the equator, shortly after anaphase onset (AO). These movements occur in association with the development of spindle interzone, the deposition of 'telophase disc' materials, the alignment of equatorial actin filaments, the dephosphorylation of myosin heavy chain (MHC), and the assembly of myosin filaments at the equator. Cytokinesis is accompanied by a phosphorylation switch on MLC_{20} from negative regulatory sites to the stimulatory Ser19, presumably enhancing myosin ATPase and assembly activity in the cleavage furrow. Completion of cytokinesis is marked by a rapid disassembly of contractile structures in the furrow region upon re-entry into interphase of the next cell cycle.