Cytokinesis

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The actomyosin contractile-ring mechanism remains the paradigm for cytokinesis after 20 years of experimental testing. Recent evidence suggests that Ca^{2+} triggers the contraction and that cell-cycle kinases regulate the timing of cytokinesis. New work is required to identify the signals from the mitotic spindle that specify the position of the furrow.

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Introduction

The division of mitotic cells by cytokinesis was observed for more than two centuries before Marsland and Landau in 1954 proposed the modern theory that the cleavage furrow forms by contraction of an equatorial band of cortical cytoplasm. At this time, Hoffmann-Berling also observed that cytokinesis shares some of the characteristics of muscle contraction, including dependence on Mg-ATP. Here, we will summarize recent efforts to elucidate how the cell orchestrates the assembly, constriction and disassembly of the contractile ring.

For the past 15 years investigators have concentrated on the 'sliding-filament contractile-ring' model of cytokinesis (Fig. 1). Throughout this review we will refer to the concepts illustrated in this figure as the 'contractile-ring model'. The contractile ring is a transient bipolar array of actin filaments with their barbed ends attached to the plasma membrane at sites around the equator of the dividing cell. The interaction of these actin filaments with bipolar myosin-II filaments applies tension to the membrane, much like the contraction of smooth muscle. Because the contractile ring is confined to a narrow equatorial band of cortex, it constricts the cell locally and pinches it in two like a purse string. Signals emanating from the poles of the mitotic spindle are thought to determine the position of this contractile ring at a point midway between two poles. Although it initially contracts with constant volume like muscle, the number of actin filaments in the contractile ring declines during the later stages of cytokinesis. A recent symposium volume [1••] and excellent reviews by Mabuchi [2], Rappaport [3], Salmon [4] and Hepler [5] summarize much of our knowledge about cytokinesis.

Although many features of the contractile-ring model are likely to be correct, we know relatively little about the biochemical mechanism of cytokinesis. For example, the inventory of structural and regulatory proteins in the contractile ring is incomplete. Nor do we know how the actin filaments attach to the membrane, become organized around the equator or disappear as the furrow contracts. In addition, there is little information on the signals from the mitotic poles or on the mechanism of recruitment of myosin-II to the contractile ring. We now have the first biochemical evidence of how the enzymes that control mitosis might determine the timing of cytokinesis, but no direct evidence for the mechanisms that regulate the contractile activity of the furrow itself.

Structure and dynamics of the contractile ring

Inventory of contractile-ring proteins

The major proteins of the contractile ring (Table 1) were identified initially by electron microscopy and fluorescent antibody staining, and new proteins have been recognized recently in contractile rings isolated by cell fractionation [6,7•]. The identity of several of the new proteins concentrated in isolated furrows remains unknown. The fact that isolated cleavage furrows contain only a subset of the proteins found in the isolated cortex is encouraging, although it is not yet clear whether the cleavage furrow preparations are pure or if any proteins are lost during purification.

We do not know whether the reported variability in the concentration of actin and myosin-II in the contractile ring (Table 1) [8] really exists or whether it arises from differences in the methods of preparation, detection or observation. One unexplored possibility for biological variability is that different antibodies may detect only a subset of the isoforms of actin [9] and myosin-II [10] and that a limited number of isoforms of actin and myosin-II are concentrated in the contractile ring.

The inner centromere proteins (INCENPs) of 135 kD and 150 kD [11•] are the first contractile-ring proteins demonstrated to originate in the mitotic apparatus. They are tightly associated with the chromosomes during metaphase, move to the continuous microtubules in the middle of the anaphase spindle and then redistribute

Abbreviations

INCENP—inner centromere protein; **MLCK**—myosin light chain kinase; **MPF**—maturation-promoting factor; **PKC**—protein kinase C; **PP**—protein phosphatase; ***sqh***—*spaghetti squash*; ***sry-α***—*serendipity-α*.

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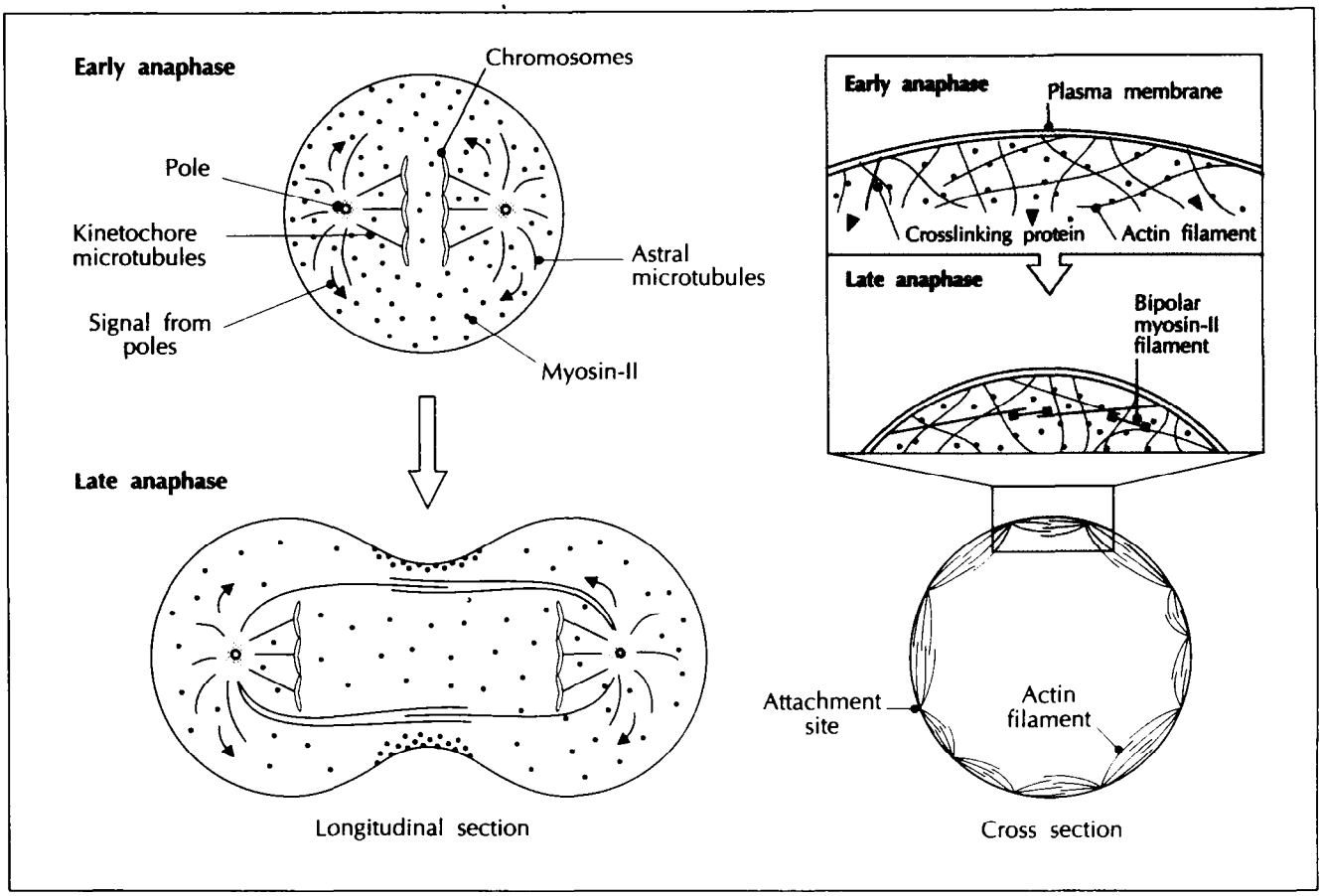

Fig. 1. The contractile-ring model of cytokinesis. In early anaphase a positive signal (indicated by arrows) travels from the poles of the mitotic spindle to the equatorial cortex where it stimulates the assembly and contraction of a ring of actin filaments and myosin-II filaments. The actin filaments are attached to the plasma membrane at their barbed ends, and so the tension in the ring constricts the equator of the cell like a purse string. The arrowheads in the cross section indicate the polarity of the actin filaments with their barbed ends at the membrane.

Table 1. Inventory of contractile-ring proteins.

Protein	Methods of identification	Concentrated in furrow?	References
Actin (43 kD)	EM, Fl-Ab, Fl-actin, Fl-phalloidin, isolation	Variable	[13–15,28•]
Myosin-II (200 kD)	Fl-Ab, Fl-myosin, isolation	Usually	[7•,8,19,22,25]
α-Actinin (100 kD)	Fl-Ab, isolation	Variable	[7•,8,79]
Filamin (280 kD)	Fl-Ab	No	[8]
Inner centromere protein (135 and 150 kD)	Fl-Ab	Yes	[11•]
Radixin (82 kD)	Fl-Ab	Yes	[20•]
Spectrin (220 kD)	Isolation	?	[7•]
68 kD	Isolation	Yes	[7•]
34 kD	Isolation	Yes	[7•]
32 kD	Isolation	Yes	[7•]
Serendipity-α	Genetic screen, Fl-Ab	Yes	[12•]
Integral membrane sialoglycoprotein (130 kD)	Fl-Ab	Yes	[80]

Ab, antibody; EM, electron microscopy; Fl, fluorescent.

to the cortex just before the furrow forms. The INCENPs share some sequence homology with actin- and tropomyosin-binding proteins, making them candidates for structural components of the contractile ring. Alternatively, they may even carry a signal from the spindle to the cortex.

Genetic analysis of cellularization in *Drosophila* has identified several genes, including *serendipity-α* (*sry-α*), that affect the hexagonal arrays of cleavage furrows that divide the embryonic syncytium into cells [12•]. The sry-α protein co-localizes with actin in the hexagonal arrays and the loss of sry-α proteins results in abnormal spacing of furrows and multinucleated cells , suggesting that sry-α might stabilize these specialized furrows. Further characterization of cellularization mutations should reveal additional components of the cleavage furrow.

The current inventory of contractile-ring proteins is surely incomplete. For example, none of the candidate regulatory proteins (myosin kinases and phosphatases) have been identified but, to our knowledge, none have been sought. These molecules are more likely to be isolated using genetic screening than any other approaches.

Organization of the contractile-ring filaments

The contractile ring consists of a very thin (0.1–0.2 μm) overlapping array of actin filaments [13,14] with opposite polarities [6,15]. This mixture of actin filament polarities is important in the contractile-ring model (Fig. 1) because the sliding-filament mechanism depends on tension generated by the interaction of bipolar myosin filaments with oppositely polarized actin filaments, as in muscle. *Dictyostelium* has a second array of actin filaments aligned parallel with the central spindle, just deep to the circumferential actin filaments of the contractile ring [16••].

In general, the actin filaments of the contractile ring are arranged circumferentially around the equator [17], but we do not know precisely how the contractile ring filaments are orientated in three dimensions. In Hela cells many are aggregated into small bundles of about 20 filaments, similar in size to microvillar bundles [18]. In *Dictyostelium*, contractile ring birefringence becomes detectable only well after furrowing commences [16••], indicating that the alignment of the actin filaments increases during the constriction of the furrow. This behavior is consistent with the contractile-ring model (Fig. 1) where tension between attachment sites confined to a narrow band around the equator would tend to align the filaments.

Studies using both light [19] (S Yonemura and T Pollard, unpublished data) and electron microscopy [15,18] suggest that myosin-II is assembled into small bipolar filaments in the contractile ring. Electron microscopy shows the putative myosin filaments interdigitated with the actin filaments, as pictured in the contractile-ring model (Fig. 1). However appealing these observations may be, more work is necessary because the immunofluorescent myosin-II spots observed in protozoa have not been confirmed to be filaments by electron microscopy and because the 13-nm filaments detected by electron microscopy in Hela cells have not been positively identified as myosin-II. Furthermore, the immunofluorescent myosin spots are not visible in all micrographs in *Dictyostelium* [16••].

Attachment of contractile ring to the plasma membrane

Electron microscopy of intact cells [17,18] and isolated furrows [6] has revealed that the actin filaments of the contractile ring are attached to the plasma membrane at many sites around the equator. In isolated furrows, they appear to be attached to the membrane at or near their barbed ends. These features are consistent with the contractile-ring model (Fig. 1) because myosin is a barbed-end-directed motor. In Hela cells, many of the actin filaments appear to radiate from well separated, electron-dense attachment plaques [18] like those in smooth muscle. Other filaments may attach at more dispersed sites, but this is not well established.

A new barbed-end actin-filament-capping protein named radixin is highly concentrated in both the cleavage furrow and adherens junctions of several cultured cell lines [20•]. This makes radixin an attractive candidate for a participant in the attachment of the contractile-ring actin filaments to the plasma membrane, although nothing is known about any molecular interactions.

Most would assume that myosin-II is associated with the contractile ring by binding to actin filaments, but new experiments suggest that it may bind independently to isolated sea urchin egg cleavage furrows [21]. Myosin-II remained concentrated in furrows extracted with high salt and ATP to dissociate actomyosin, or with gelsolin to depolymerize the actin filaments. The nature of the putative attachment between the myosin-II and the plasma membrane remains to be discovered.

Assembly of the contractile ring

The formation of the contractile ring is part of a dramatic reorganization of the whole cytoskeleton during mitosis that begins long before cytokinesis. These changes were observed originally in static images of cells stained with antibodies to actin and myosin-II, but have been documented most dramatically in cells injected with fluorescent derivatives of actin or myosin-II regulatory light chains [22,23••,24].

The assembly of myosin-II in the contractile ring is especially important for the contractile-ring model because the accumulation of myosin is one of the earliest events and the force produced by myosin–actin interactions may even align the filaments of the ring. In cultured vertebrate cells, the stress fibers break down during prophase and the constituent actin and myosin-II are dispersed throughout the cytoplasm until very early in anaphase when the myosin-II becomes concentrated in the cortex, especially around the equator where the furrow will form [22,25]. In *Dictyostelium* the myosin-II that was concentrated in the cortex during interphase becomes dispersed throughout the cytoplasm at prophase. At the metaphase–anaphase transition, myosin-II accumulates first in the cortex and then appears to redistribute within the cortex to the site of the presumptive cleavage furrow [19,26]. In marine eggs the myosin-II remains diffusely distributed in the cytoplasm during prophase and metaphase before redistributing to the cleavage furrow at anaphase [27].

Essentially nothing is known about the mechanisms underlying the accumulation of myosin-II in the contractile ring. It is not even clear whether the myosin-II moves to the equator as individual molecules or as oligomers, or whether the process is active or driven by diffusion.

The well organized appearance of the contractile ring compared with the rest of the cortex has led many to assume that it forms *de novo* from actin and myosin monomers, but new experiments with phalloidin suggest that some of the actin filaments of the contractile ring are recycled intact from other parts of the cell. Actin filaments labeled and stabilized *in vivo* by microinjection of fluorescent phalloidin were observed to move from other parts of interphase cells to participate in the contractile ring [28•]. Actin filaments labeled and stabilized with fluorescent phalloidin *in vitro* redistribute in a two-step process when injected into dividing cells [23••]. Filaments accumulate in the cortex late in metaphase and then migrate by an unknown mechanism within the cortex toward the cleavage furrow at anaphase. This is an example of a cortical-flow phenomenon [29].

In contrast to the view that the contractile ring forms by reorganization of relatively stable preexisting actin filaments, drug studies provide evidence that the actin filaments of the contractile ring are dynamic. Cytochalasin (which partially blocks the barbed end of actin filaments and promotes the conversion of ATP–actin monomers to ADP–actin [30]) causes the contractile ring to break [31] and the cleavage furrow to regress [32]. On the other hand, phalloidin (a stabilizer of actin filaments) can stop the progression of furrowing [33]. No one knows the mechanisms of these drug effects at the cellular level, but purified actin *in vitro* would not react this way. Therefore, a naturally occurring, relatively rapid turnover of the contractile ring actin filaments is required. The divergent conclusions regarding the stability of actin filaments in the contractile ring should be reconciled by photobleaching or photoactivation studies on live cells.

Disassembly of the contractile ring

The contractile ring undergoes an isovolumetric contraction during the early stages of furrowing and then disassembles during the completion of cleavage [17]. This is consistent with the sliding-filament contractile-ring model but, as yet, little is known about the details of the process. The number of actin filaments in each contractile-ring bundle remains constant at about 20 as the furrow constricts by up to 90% [18]. This observation is easier to reconcile with a disassembly mechanism in which each actin filament shortens progressively, rather than one where whole filaments are lost and the remaining filaments regroup into new bundles of 20.

Experiments with *Dictyostelium* expressing myosin-II with a truncated tail provide evidence for the importance of myosin filament disassembly in the function of the contractile ring [34•]. The truncation of the terminal 34 kD of the tail removes phosphorylation sites required for disassembling myosin-II filaments. Cells expressing only truncated myosin-II can divide by cytokinesis in suspension like wildtype cells, but only imperfectly, yielding daughters of different sizes with clumps of myosin-II in the cytoplasm.

Tests for functions of contractile-ring proteins

That myosin-II participates as the motor for cytokinesis has been established by both injection of inhibitory antibodies into Starfish [35] and *Drosophila* [36], and genetic depletion of the myosin-II heavy chain from *Dictyostelium* [37,38] and of the myosin regulatory light chain from *Drosophila* [39••]. *Dictyostelium* lacking the myosin-II heavy chain can still extend pseudopods, round up during mitosis and complete nuclear division, but cannot form a cleavage furrow. On a solid substrate these cells can divide into daughter cells that literally crawl away from each other, but this alternate cleavage process is unreliable and fails in suspended cells. This traction-mediated cytofission (also called 'amitosis') may resemble cytokinesis in cells prior to the evolution of myosin-II.

Mutations in the gene for the regulatory light chain of *Drosophila* cytoplasmic myosin-II (*spaghetti squash*, *sqh*) provide the first evidence that myosin-II is essential for cytokinesis [39••]. Mutants with a P-element insertion in the 5'non-translated region of *sqh* have low levels of regulatory light chain mRNA and enormous polyploid larval cells, resulting from a failure of cytokinesis at the time that the maternal supply of regulatory light chain becomes limiting.

Disruption of the gene for the myosin-II heavy chain in *Saccharomyces cerevisiae*, *MYO1*, results in incomplete cytokinesis and aberrant nuclear migration [40]. A disruption of the putative tail domain of *MYO1* leads to slightly defective budding patterns and clear defects in chitin deposition and cytokinesis [41•]. These defects may be connected to the localization of uncharacterized 10-nm filaments and myosin at the mother–bud junction [42], but the relationship of these events in budding yeast to cytokinesis in other eukaryotic cells is not clear. On the other hand, fission yeast have a contractile actin filament ring at the site of septation [43•,44•] and so, in spite of morphological differences, genetic analysis of cytokinesis in yeast may be informative about some aspects of cytokinesis in other cells.

Mechanisms regulating cytokinesis

Signals regulating the position of the cleavage furrow

It has long been appreciated that formation of the cleavage furrow requires both a signal from the mitotic apparatus and a responsive cortex (reviewed in [3]), and so a minimal signalling mechanism must include the following: a signal source; a mobile signal (most likely a molecule); a cortical receptor; and one or more cortical signal transduction steps that lead to the assembly of the contractile ring and constriction of the furrow. We expect that the activities of several of these components are under cell-cycle control (see below).

Research on the signalling mechanism has been severely limited because the only available assay for these reactions is the behavior of live cells. The development of an *in vitro* assay or even bioassays for the individual steps would have a profound impact on research. Tissue culture cells permeabilized in anaphase can continue to constrict established cleavage furrows in MgATP with micromolar Ca^{2+} [45], but these model systems have not yet been exploited to investigate the signals that establish the furrow.

To illustrate the challenge of characterizing a complex cascade in a live cell, the simple question of when the signal emerges from the mitotic spindle should be considered. The mitotic apparatus is not seen to stimulate a furrow until anaphase. One possible explanation for this is that the signal actually emerges at anaphase. Alternatively, the signal could emerge earlier, but could fail to stimulate cleavage until the cortex becomes responsive at anaphase. These alternatives are difficult to distinguish in live cells, but new evidence suggests that the signal is present early in prophase [46]. In binucleate echinoderm eggs where the cycle of one nucleus is caused to lag behind the other by ultraviolet irradiation, either prophase asters or prometaphase spindle asters of the lagging nucleus are capable of inducing cleavage at the time that the mitotic apparatus of the leading nucleus enters anaphase.

It is generally believed that the asters of the mitotic spindle specify the position of the cleavage furrow. (Interested readers should consult [3] for the wealth of experimental details.) This signalling mechanism determines the position of the cleavage furrow midway between the spindle poles and perpendicular to the long axis of the spindle, thus ensuring that the daughter nuclei are separated by the cleavage process. The poles and their astral array of microtubules are regarded as the source of the signal rather than components of the central spindle, such as the chromosomes, because furrows form midway between two poles even when no chromosomes are present.

The molecular nature of the signal from the spindle asters remains a mystery, although several of its features are known [3]. Because asters induce cytochalasin-sensitive tension in the adjacent cell surface [47], the signal is thought to activate rather than inhibit contraction. This conclusion is supported by two recent efforts to model cytokinesis computationally [48,49], although some investigators favor a mechanism where the signal relaxes the poles [50]. Spindles can induce multiple furrows if repositioned at succeeding time points close to the cell surface with a needle [3]. The polar signal becomes fixed or transduced into a regenerating cortical signal after only 1 min exposure of the cortex to a mitotic apparatus. Furrowing commences after a lag of 4 min and propagates around the equator even in the absence of the spindle. The signal moves from the poles to the cortex at about $7\,\mu m\,min^{-1}$. Because the astral microtubules are required, the signal may even be carried physically toward the cortex along the microtubules, although the astral microtubules do not actually penetrate the equatorial cortex [51].

Given the lack of information on the signal and the limitations of the live-cell assays, little is certain about the downstream steps including the cortical receptor(s) or the mechanisms that transduce the signal into the cortical response. In the following sections we will consider the evidence for some of these downstream components.

Calcium

Calcium has long been suspected to be a second messenger regulating mitosis and cytokinesis [2,5] but only recently has it been possible to demonstrate a close temporal and spatial connection between a cytoplasmic calcium transient and propagation of the cleavage furrow. Following injection of the photoprotein aequorin into medaka eggs, Fluck, Miller and Jaffe [52••] imaged a wave of micromolar calcium advancing slowly, just ahead of the cleavage furrow, at a rate of about $0.5\,\mu m\,sec^{-1}$. Because Ca^{2+} is the only documented potential second messenger for cytokinesis and because it has been difficult to record reproducible Ca^{2+} transients in dividing cells [5], it is exceptionally important to confirm the observations of Fluck *et al.* in different cells and to make some mechanistic connections to the upstream signal from the mitotic spindle.

The proposal that a Ca^{2+} wave triggers cytokinesis has several attractive features. First, a Ca^{2+} wave could be the positive signal that activates myosin light chain kinase (see below). Second, Ca^{2+} waves can be self-propagating, which might explain how the polar signal becomes fixed in and spreads across the cortex. This Ca^{2+} wave may have a novel mechanism, because it moves so slowly and propagates linearly. Third, use of Ca^{2+} as a second messenger explains a number of earlier observations [5]. For example, it was found that exposure of dividing cells to the Ca^{2+} ionophore A23187 accelerated the appearance and rate of propagation of the cleavage furrow. Calcium injection can also stimulate cytokinesis locally [53] and injection of EGTA can inhibit cytokinesis [54]. Micromolar amounts of Ca^{2+} are optimal for furrow constriction in permeabilized tissue-culture cells [45].

Myosin light chain kinase

Although there is a lack of direct evidence, three factors argue for a pivotal role of myosin light chain kinase (MLCK) in activating the contraction of the cleavage furrow. First, cytoplasmic and smooth muscle myosin-II share the same regulatory light chain. Second, strong physiological evidence has established that phosphorylation of Ser19 (and Thr18) of the regulatory light chain is linked with the initiation of contraction in smooth muscle [55•]. We refer to these residues as 'activating sites' (Fig. 2). The MLCK that catalyzes this reaction is activated by calcium–calmodulin. Third, there is growing evidence that Ca^{2+} acts as a second messenger in cytokinesis (see above), thus providing a biochemical signal to activate MLCK. In addition, phosphorylation of the regulatory light chain is one factor regulating the polymerization of cytoplasmic myosin and might play a role in the assembly or disassembly of the contractile ring [56].

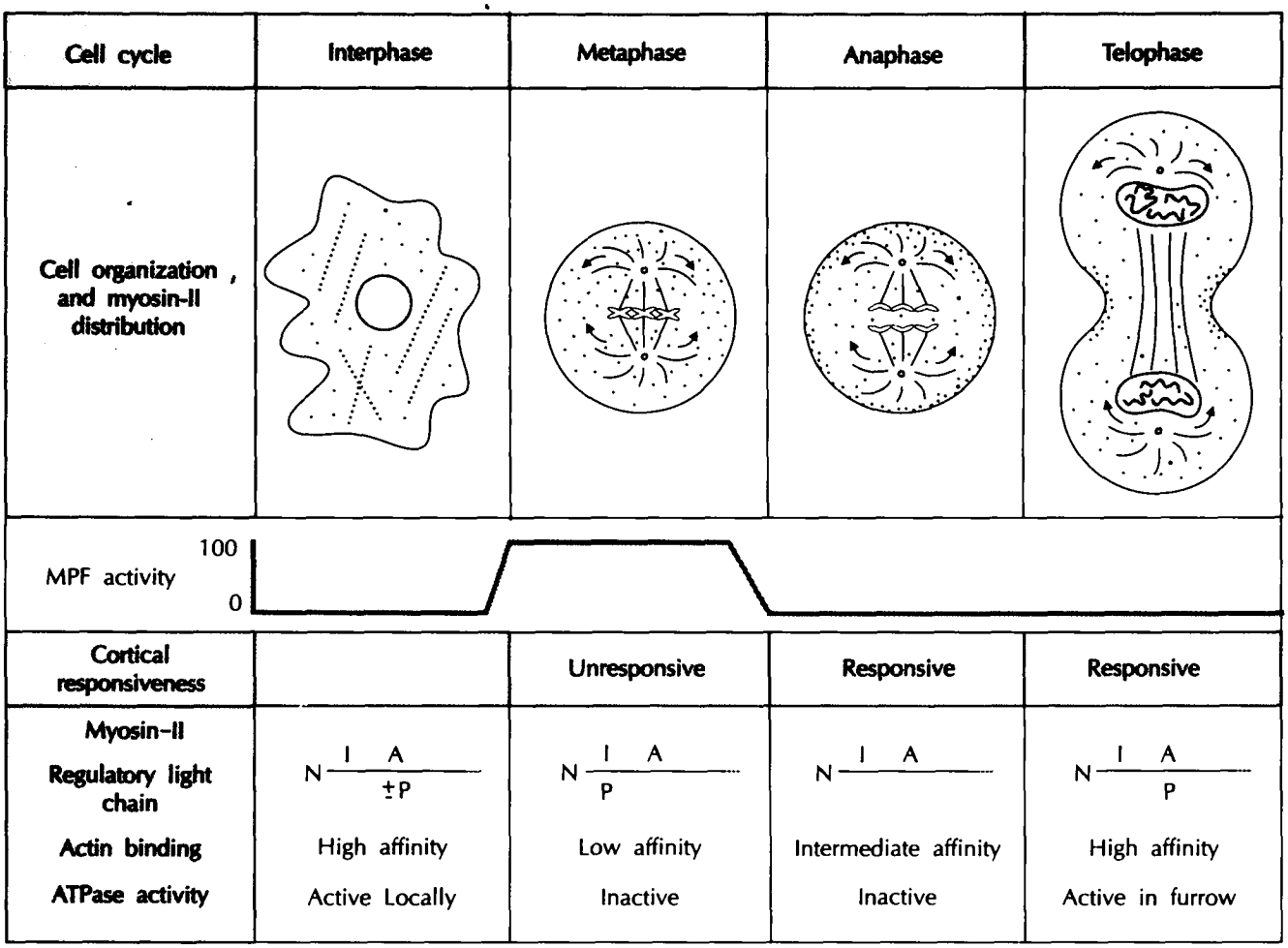

Fig. 2. A hypothetical model for how maturation-promoting factor (MPF) might regulate the distribution of myosin-II and the timing of cytokinesis. The various stages of mitosis are shown in relation to the activity of MPF. The bottom panel gives the predicted properties of myosin-II. The first row shows diagramatically the amino terminus of the regulatory light chain with the inhibitory (I) and activating (A) phosphorylation (P) sites. At interphase, the myosin-II (shown as black dots) is largely associated with cytoplasmic structures composed of actin filaments, including stress fibers. Some of the myosin-II is activated locally by myosin light chain kinase (MLCK) to stimulate contractile activities. As the cell enters mitosis, MPF phosphorylates inhibitory sites on the regulatory light chains of myosin-II. Phosphatases including protein phosphatase (PP)1 remove phosphates from the activating site on the light chain. The myosin-II then has a low affinity for actin and dissociates from stress fibers, which also fall apart at this time. Positive signals from the poles of the mitotic apparatus (arrows) fail to activate the cortex, because the myosin-II is inhibited globally by MPF. At anaphase, the MPF activity declines and phosphatases (perhaps PP2A) dephosphorylate the inhibitory sites on the light chain. The myosin-II has higher affinity for actin filaments and becomes concentrated in the actin-rich cortex. This unphosphorylated myosin-II is inactive but is responsive to signals from the poles, as it can be phosphorylated on the activating sites by MLCK. It is postulated that the mitotic signal releases Ca^{2+} around the equator and activates the MLCK, leading to the local contraction that cleaves the cell in two.

The characterization of MLCK mutations deserves to be given a high priority, as the initial studies by microinjection methods have not resolved the role of MLCK in cytokinesis. Fishkind *et al.* [57•] microinjected a constitutively active fragment of MLCK into mitotic cells. This delayed mitosis and stimulated surface activities but did not interfere with cytokinesis.

Regulation of the timing of cytokinesis by phosphorylation

Recent observations suggest that maturation-promoting factor (MPF), the enzyme that catalyzes entry of eukaryotic cells into mitosis and meiosis [58], also coordinates the reorganization of the cytoskeleton during mitosis. MPF consists of a regulatory cyclin and the $p34^{cdc2}$ kinase. For this discussion, the most important feature of MPF is its cycle of activity. MPF is activated in prophase and declines rapidly at the metaphase–anaphase junction because of cyclin proteolysis. Both microtubule dynamics [59,60] and intermediate filament arrays (see the review by Eriksson, Opal and Goldman, this issue, pp 99–104) are regulated by MPF phosphorylation during mitosis. We will focus here and in the next section on new evidence that the cyclic activation of MPF and specific phosphatases may control the timing of cytokinesis.

MPF phosphorylates the regulatory light chain of myosin-II (Satterwhite LL, Minshull J, Hunt T, Cisek L, Corden J, Pollard TD: *J Cell Biol* [abstract] 1990, **111**:135a) [61•] on residues known to inhibit the actin-activated myosin ATPase *in vitro* [62]. These inhibitory sites (Ser1, Ser2 and Thr9; Fig. 2) are also phosphorylated by Ca^{2+}-phospholipid-dependent protein kinase C (PKC). Phos-

phorylation of these residues by PKC inhibits the actin-activated ATPase of smooth muscle myosin previously phosphorylated on Ser19 and inhibits the phosphorylation of Ser19 by MLCK [62,63]. Phosphorylation of the inhibitory sites by PKC is also correlated with relaxation of contracted smooth muscle [55•].

The myosin-II light chains are phosphorylated at the inhibitory sites in mitotic lysates of *Xenopus* eggs (LL Satterwhite and TD Pollard, unpublished data). Inhibition of myosin-II by MPF phosphorylation of light chains during prophase and metaphase could delay cytokinesis until chromosome segregation begins in anaphase and thus provide the cell with a timing mechanism to prevent premature cytokinesis. In this way, MPF may contribute to the lack of cortical responsiveness to mitotic signals during prophase and metaphase. Because phosphorylation of the inhibitory sites lowers the affinity of myosin-II for actin filaments *in vitro* [55•], the observed changes in myosin-II distribution during mitosis could result from cyclic changes in the affinity of myosin for actin filaments concentrated in the cortex. However, this redistribution is surely more complex because the heavy meromyosin fragment of *Dictyostelium* myosin-II possessing the regulatory light chain does not localize to the equatorial cortex at anaphase [64••].

Bement and Capco [65•] have obtained evidence that activation of PKC promotes the contraction of the cortex that closes wounds in *Xenopus* eggs. They argue that this contraction might be a model for cytokinesis and that PKC might stimulate rather than inhibit cytokinesis. Further investigation is required to establish whether wound healing and cytokinesis are regulated in the same way.

Interphase fibroblasts round up and enter mitosis prematurely if injected with cyclin-p34 kinase [66•], indicating that MPF may also influence the organization of actin filaments during mitosis. One actin-binding protein, caldesmon, is phosphorylated in mitotic cells at the same sites phosphorylated by MPF *in vitro* [67••]. Phosphorylation causes caldesmon to dissociate from actin filaments [68••], but not enough is known about the functions of caldesmon to know how its phosphorylation might contribute to the reorganization of the cytoskeleton.

MPF is not the only cell-cycle-regulated kinase active during mitosis. For example, the nimA protein kinase is also required for *Aspergillus* to enter mitosis [69]. This, together with other unidentified kinases, as well as kinases known to be activated downstream of MPF [58], is a potential regulator of all the components of the signalling pathway.

Regulation of the timing of cytokinesis by phosphatases

Recent genetic and biochemical studies suggest that protein phosphatase (PP)2A regulates entry into mitosis and that PP1 is essential for exit from mitosis. PP2A, a component of the MPF inhibitor INH [70] inhibited entry into mitosis. Inhibition of PP2A in *Xenopus* extracts activated $p34^{cdc2}$ kinase prematurely [71]. In genetic studies, mutations in *Schizosaccharomyces pombe* and *Aspergillus nidulans* PP1 genes [72–74] block exit from mitosis. Disruption mutants in *S. pombe* PP2A genes exhibit premature mitosis [75].

A possible connection between cytokinesis and these cell-cycle-specific requirements for PP1 and PP2A is the activities of these enzymes as myosin-II light chain phosphatases *in vitro* [76]. PP1 dephosphorylates the activating site of the light chain at a higher rate than the inhibitory site, whereas PP2A dephosphorylates preferentially the inhibitory sites. Accordingly, myosin light chains were dephosphorylated at the activating site in fibroblasts injected with PP1 at the same time that actin filament bundles disappeared [77••].

At prophase, PP1 may dephosphorylate the activating site on the regulatory light chain as MPF phosphorylates the inhibitory site, whereas, at anaphase, PP2A may dephosphorylate the inhibitory site as MLCK phosphorylates the activating site to initiate contraction of the cleavage furrow. These complementary kinase and phosphatase activities could increase the rate of phosphorylation by both kinases, because phosphate at one site on the light chain reduces the rate of phosphorylation by a second kinase at the second site [62].

Prospects

The contractile-ring model (Fig. 1) accounts for much of what we know about cytokinesis and is likely to remain the paradigm for experimentation in the near future. Nevertheless, it would not be surprising if some of its details were revised by new information (see, for example, a completely different view [78]). Much work on the contractile ring is needed to complete the molecular inventory, resolve its three-dimensional ultrastructure and document the reactions required for its assembly, contraction and disassembly. The conclusions from these molecular studies will need to be tested by future mechanical modeling [48,49].

Elucidating the molecular control mechanisms specifying the position, timing and activity of the cleavage furrow represents a greater challenge than understanding the molecular mechanics. Not the least of the problems is the lack of good assays for the individual molecules and reactions. Fortunately, genetic studies of flies and yeast offer promising new angles of attack.

The ideas embodied in Fig. 2 provide a possible biochemical foothold on one part of the regulation puzzle; the question of cortical responsiveness. This oversimplified hypothesis focuses on myosin-II, because it appears to be a central player in cytokinesis. It is essential for cytokinesis, and the tension generated by its interaction with actin filaments may even contribute to the assembly of the contractile ring. Currently, myosin-II is the only molecular candidate for a role in the final common pathway for the signals from the mitotic spindle. If the proposed role of MPF in regulating the time of cytokinesis is confirmed, it may be possible to work outward experimentally from this step to identify both the upstream signals and the downstream mechanical output of the system.

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